TRANSFUSION

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Physiological Basis for Transfusion Therapy in Hemorrhagic Disorders:

A Critical Review

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The theme of this issue is the preservation of plasma clotting factors. Dr. Aggeler was invited to prepare an editorial on this subject. He became so engrossed that before he knew it his editorial had grown to the proportions of this review which is presented as an "editorial review." (Editor)

THE USE of blood or plasma to alleviate bleeding due to a deficiency of one or several known clotting factors is common practice. Abnormal bleeding may occur because of thrombocytopenia, thrombocytopathia or a deficiency of fibrinogen, prothrombin, PTA, or factors V, VII, VIII, IX, or X.* In Von Willebrand's disease there may be a deficiency both of factor VIII and of a plasma factor which controls the bleeding time.[71] Some types of congenital thrombocytopenia appear to be due to the lack of a plasma factor which is necessary for the production of platelets.[88] Bleeding may also occur because of complex deficiencies of platelets and clotting factors in the newborn, in diseases of the liver, in multiple myeloma, in Waldenstrom's macroglobulinemia, in hemolytic transfusion reactions, in certain obstetrical complications, such as abruptio placentae, amniotic fluid embolism, self induced abortion, and dead fetus, in some patients with carcinoma of the prostate, pancreas, stomach and elsewhere, and in certain surgical operations, particularly on the brain, lung, or prostate. The bleeding due to deficient clotting factors in obstructive jaundice, malabsorption syndromes, some cases of hemorrhagic disease of the newborn, and following the use of dicumarol and related compounds is best treated with vitamin K. In other conditions reliance must be placed on transfusion of normal plasma or the use of concentrated clotting factor extracts.

Rational therapy of bleeding disorders with plasma requires a knowledge of the *in vitro* and *in vivo* survival of clotting factors, and of the minimum concentrations

*PTA—Plasma Thromboplastin Antecedent.
Factor V—proaccelrin, labile factor, Ac globulin.
Factor VII—proconvertin, stable factor, serum prothrombin conversion accelerator (SPCA), co-thromboplastin.
Factor VIII—Antihemophilic factor (AHF), antihemophilic globulin (AHG), Antihemophilic factor A (AHF-A), alpha-prothromboplastin.
Factor IX—Plasma thromboplastin component (PTC), Antihemophilic factor B (AHF-B), beta-prothromboplastin, Christmas factor.
Factor X—Stuart factor, Stuart-Prower factor.

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required to produce hemostasis. Under different conditions of collection and storage of plasma there is a variable loss of platelets and clotting factors. The half-life *in vivo* of some clotting factors is only a few hours, while that of others is several days. In some hemorrhagic states only one factor need be replaced, while in others multiple deficiencies must be treated. The "minimum hemostatic level" may vary depending on which factors are deficient and upon a variety of other conditioning circumstances.

In Vitro Survival of Platelets and Clotting Factors

The survival of platelets and of several coagulation factors is influenced by the technic of blood collection, the duration and temperature of storage, and the collecting medium. Donor blood may be collected in glass, silicone-lined or plastic containers, by gravity or vacuum, using acid-citrate-dextrose (ACD) mixture, trisodium citrate, disodium ethylenediaminetetraacetate (EDTA), or heparin as anticoagulant. The plasma may be separated immediately, or after storage; it may be used within a few hours, or frozen, lyophilized, or stored in the liquid state.

Platelets: Platelets may be damaged in preparation and storage of blood or plasma. The efficacy of a platelet preparation can be assayed reliably only by determining the net rise in platelets and the duration of their survival after transfusion into a suitable recipient. There is no method for distinguishing viable and nonviable platelets, *in vitro*.

The circulating yield of platelets after various transfusion technics was given by Gardner[38] as follows:

	Per cent
Whole blood, direct, silicone, multiple syringe	80-90
Whole blood, fresh (1 hour), plastic bag	75-80
Platelet rich plasma, plastic bag	60-65
Platelet concentrates, plastic bag	50-55
Whole blood, fresh gravity collection, glass vessel	10-15
Stored blood, glass vessel (24 hours)	0

Gardner found no difference in platelet viability, whether ACD solution or sodium EDTA was used as anticoagulant. Baldini, *et al.*,[12] however, felt that EDTA is preferable, at least in the preparation of platelet concentrates. As noted above and confirmed by Mustard and Walker,[70] silicone-coated and plastic surfaces appear to minimize platelet loss during collection. It has been emphasized by several observers [3, 38, 0] that incipient blood coagulation caused by slow and inadequate mixing of blood and anticoagulant, the introduction of tissue juices into the specimen by imperfect venipuncture, and prolonged centrifugation will markedly shorten platelet yield and survival. When strict attention has been paid to these factors it has been found that platelets survived equally well *in vitro* and *in vivo*, whether the blood is drawn into glass bottles or plastic bags.[57] Platelets do not survive well in stored blood regardless of the type of container or collection technic. It appears, therefore, that nonwettable surfaces are beneficial because they minimize procoagulating influences caused by faulty collection technic. While perhaps unnecessary under ideal conditions, plastic containers would still seem desirable for collection and storage of blood for platelet transfusions. Most reports emphasize that the increased blood-air interface caused by frothing when vacuum bottles are used produces greater loss of platelets and clotting factors than blood collected by gravity. However, this has recently been disputed.[20, 69, 70]

Earlier attempts to isolate and preserve platelets in artificial media were disappointing. However, recent experiments of Tullis, *et al.*,[94] using platelet suspensions stored in gelatin for as long as two years appear encouraging. The suspensions were prepared by a special technic, which involves rapid separation and repeated washing of the platelets to free them of plasma proteins. On the other hand, Baldini, *et al.*,[12]

found poor viability of platelets washed in platelet poor plasma and resuspended in gelatin or saline. They emphasize the need for plasma in the suspending medium in order to preserve viability of the platelets. Klein, *et al.*, have also reported temporary improvement in hemostasis following the use of lyophilized[58] and of stored frozen platelet preparations.[59] However, Jackson, *et al.*,[51] and Hjort, *et al.*,[45] found that, in contrast to fresh viable platelets, such preparations produced no increase in circulating platelet levels and no decrease in the output of red cells in thoracic duct lymph of irradiated thrombocytopenic dogs. Cronkite and Jackson[25] recently published an excellent critical review on the use of platelet transfusions in hemorrhagic diseases. In the light of present knowledge, whole blood or platelet rich plasma, drawn by gravity into siliconed or plastic containers, with sodium EDTA as anticoagulant, and transfused within three hours appears to be the most practical preparation for general use.

Factors V and VIII: The principal clotting components which deteriorate during the storage of blood bank* plasma are factors V and VIII.[9, 10, 17, 20, 21, 29, 30, 32, 39, 41, 80-84] An immediate loss of as much as 25 per cent of factor V and 40 per cent of factor VIII, may be caused by failure to maintain low temperatures during centrifugation and by incipient clotting of the blood in the collection bottle. This loss can be entirely eliminated by proper technic.[20, 80, 72]

Opinion varies regarding the disappearance rate of factors V and VIII from bank blood stored at 4 C. Soulier, *et al.*,[90] found 10 to 25 per cent of factor V remaining after one week. Kettenborg, *et al.*,[54] found 20 per cent after one week and 3 per cent after two, while Rapaport[78] found 81 per cent after five to nine days, 79 per cent after ten to 14 days, and 56 per cent after 18 to 22 days. Similarly in regard to factor VIII, Kettenborg[54] found 25 per cent of normal in one week and 20 per cent in two. Brinkhous, *et al.*,[21] found 70 per cent in one week, 57 per cent in two, and 37 per cent in three. Rapaport, *et al.*,[78] found a mean value of 55 per cent in five to seven days, 35 per cent in 12 to 14 days, and 30 per cent in 18 to 22 days.

Blood bank plasma separated within six hours of drawing shows a gradual loss of factor VIII activity even when stored at —20 C. Penick and Brinkhous[72] found that the deterioration of factor VIII proceeded at different rates in different plasmas. They observed an average loss of approximately half the activity within four weeks, but thereafter the deterioration proceeded at a much slower rate. Pool and Robinson[74] assayed factor VIII in 25 units of blood bank plasma stored for two to three months at —20 C. They found 0 to 25 per cent loss in three units, 25-50 per cent loss in seven units, and 50-75 per cent loss in 15 units. Bradlow, *et al.*,[20] found 50 per cent loss of factor VIII in one of five specimens stored for two weeks at —40 C. Two specimens stored at —20 C. or —40 C. for 77 to 132 days lost 20 per cent of their original content. Rapaport,[80] in a study of 16 single units of fresh frozen plasma prepared by the Los Angeles Red Cross Blood Center, found little loss of factor VIII during preparation, and only an average loss of 20 per cent after storage at —20 C. for one year. Despite these findings it appears unwise to rely on random units of fresh plasma stored in the frozen state in blood banks for more than two or three weeks, unless actual assays for factor VIII indicate that procedures of individual blood banks permit longer storage.

Pool and Robinson[74] emphasized the importance of dissolving all cold precipitated fibrinogen by constant agitation of the plasma during thawing at 37 C. Since the concentration of factor VIII in the precipitate is much higher than in clear plasma,

* Collected with plastic tubing into uncoated evacuated glass bottles containing one part ACD solution (1.32 per cent sodium citrate, 0.48 per cent citric acid, and 0.47 per cent dextrose) in the ratio of 1 part to 4 parts of blood.

failure to dissolve it will affect the results of *in vitro* assays on the specimen and will also lower its yield of factor VIII on transfusion.

Information is lacking regarding the stability of factor V in freshly separated blood bank plasma kept in the frozen state for prolonged periods. Our own experience would indicate that it is relatively stable for at least two or three weeks. Plasma separated from bank blood after periods of storage up to 21 days and frozen will show further loss of factor V and VIII activity on continued storage at —20 C.

It should be emphasized that storage of plasma in the freezing compartment of a refrigerator is not adequate for the preservation of clotting factors. Even storage at —20 C. may not be optimal. As suggested by Anstall, *et al.*,[9, 10] —30 C. may be superior either to —15 C. or —50 C. It is possible that some of the differences in storage stability of plasma clotting factors in the frozen state, reported by different laboratories, may be due to variations in storage temperature. One wonders how often the term "—20 C." means that the plasma was really stored in a deep freeze at a temperature somewhere between —10 and —70 C. Another subject which has received insufficient attention is the speed of freezing. Bradlow, *et al.*,[20] suggested the desirability of very rapid freezing at —40 C. but did not actually measure the effect of this particular step in their procedure. Blombäck[17] found that purified factor VIII preparations showed no change in potency when rapidly frozen at —25 C. to —70 C., but lost 50 to 75 per cent of their activity when slowly frozen at —10 C. to —15 C. The speed and temperature of freezing and thawing may be more important than the temperature of storage in the preservation of factor VIII.

Pooled fresh plasma, collected in trisodium citrate or ACD solution, shell frozen within six hours of drawing, and lyophilized within 48 hours, may retain full potency of factor VIII or may show an immediate loss of as great as 50 per cent.[20, 72, 74] There is no adequate explanation for the irregular losses which occur in some lots. Once prepared, however, lyophilized plasma is remarkably stable for several years.

The factor VIII level in normal blood varies from 50 to 200 per cent,[73] so that its ultimate yield in any preparation will depend to a large extent on the donor. It would be advantageous, although perhaps not practical, to use plasma only from donors with high levels in the treatment of factor VIII deficiency.

There are some discrepancies between the results of studies on the *in vitro* storage characteristics of factors V and VIII in blood bank plasma and in plasma used in carefully conducted laboratory experiments. Because of more careful handling of the blood in such experiments, many conditions which would contribute to the loss of these factors can be eliminated. On the other hand the use of trisodium citrate, rather than ACD solution may cause more rapid deterioration of factor VIII. Pool and Robinson[74] in a study of trisodium citrate plasma from a single donor, stored at —20 C. for periods up to one year, found a logarithmic relationship between factor VIII concentration and duration of storage, with a half disappearance time of 16 weeks. Fantl and Marr[32] found that plasma, drawn in trisodium citrate had a *p*H of 8 to 9, and that factor V and VIII activity totally disappeared when the plasma was stored in cotton stoppered glass tubes at room temperature for one month. This loss could be largely prevented by adjusting the *p*H to 6.3 ± 0.4. Similarly, Wolf[99] found good preservation of factor VIII at 4 C. for 18 hours in trisodium citrate plasma whose *pH* was adjusted between 6.2 and 8.0. However, in a group of untreated specimens, whose *p*H was found to be 7.9 to 8.1, there was a loss of 35 to 55 per cent of factor

VIII in three days and of 50 to 80 per cent in five days. Because this alkaline shift does not occur when ACD solution is employed, it is a better anticoagulant than trisodium citrate for use in the preservation of factor VIII.

Fahey, Ware and Seegers[30] found little loss of factor V in citrated plasma stored for one week at 4 C. These findings are comparable to those of Rapaport, *et al.*,[78] who used ACD plasma. This may indicate that the *p*H on storage is not as critical with factor V as with factor VIII.

Other Clotting Factors: All factors other than V and VIII are stable in blood bank plasma under the usual conditions of storage. Fahey, Ware and Seegers[30] found prothrombin completely stable in citrated plasma at 4 C., for 15 months at −25 C., or lyophilized for six years. We have found no significant loss of factors VII and IX in excess blood bank plasma stored at −20 C. for many months. Geratz and Graham[39] found an average of 95 per cent factor IX activity in blood bank plasma prepared from whole blood stored at 4 C. for three weeks and then frozen and stored at −20 C. for periods ranging from a few days to four months. Lyophilized plasma retained 68 per cent factor IX activity when stored at −20 C. for four years, and 30 per cent activity when stored at room temperature for six to eight years. Graham[41] found 91 per cent factor X activity in pooled blood bank plasma stored at 4 C. for 21 days and at −20 C. for 50 days. Duckert[29] stated that the activity of factor X was over 60 per cent in stored bank blood.

Because of the formation of an activation product[97] by the combination of PTA and Hageman factors, under the influence of glass surfaces and freezing, it is impossible to determine the storage stability of PTA in blood bank plasma by present *in vitro* assay methods. In acid-citrate, platelet-free plasma, prepared with siliconized equipment, Rapaport[81] found no loss at −20 C. storage for several weeks. On the basis of transfusion experiments Rosenthal[83, 84] states that there is no loss of PTA activity in lyophilized plasma, or in blood bank plasma separated from the cells within ten days and stored at −20 C. for at least one year.

In Vivo Survival of Platelets and Clotting Factors

Platelets: Platelet survival *in vivo* depends largely on the recipient. In normal subjects and in patients with aplastic anemia, not previously transfused, the lifespan of viable platelets is five to 11 days.[1, 2, 62] In idiopathic thrombocytopenic purpura and in patients previously immunized by repeated transfusions it may be only minutes.

Disappearance Rates of Clotting Factors; General Considerations: Before considering the fate of transfused clotting components it is necessary to review some general principles involved in the interpretation of disappearance curves. The concentration of a protein remaining in the circulation after transfusion, plotted against time on a semi-logarithmic scale, assumes the form of a single or double exponential disappearance curve. The former implies that all of the injected protein has remained in the intravascular compartment. When a double exponential type of curve is found, the early, rapidly falling portion of the curve is frequently interpreted as due to escape of the protein into an extravascular pool. Because disappearance curves of the latter type have been found for several clotting factors, after transfusion of plasma or concentrated extracts (Fig. 1), it has been argued that there may be extravascular pools for all clotting components. However, the existence of such pools for factors other than fibrinogen has not been proven, and it is therefore impossible, on the basis of presently available data, to determine whether the first exponential represents transfer to an extravascular compartment,

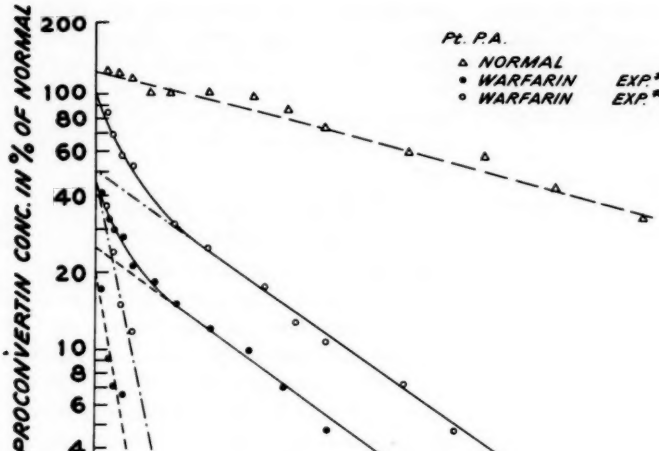

FIG. 1. Disappearance curves following injection of a concentrated factor VII extract to a normal subject. The upper curve was obtained before administration of Warfarin. Since it has but a single exponential the half disappearance time and half-life are identical — 2:30 minutes. The two lower double exponential curves were found after injection of the extract in different amounts when the factor VII concentration had been reduced to 13-14 per cent after the administration of Warfarin. The half disappearance time was 30 to 35 minutes, and the biologic half-life, in both experiments, was 85 minutes. (From Hoag, *et al.*[48])

endothelial adsorption, a change in reactivity of the factor, the influence of a cofactor or inhibitor, rapid metabolism of partially denatured protein, or some other mechanism. However, for the sake of convenience, the first exponential of such disappearance curves will be referred to in this review as indicating the *apparent* rate of diffusion of a factor into extravascular pools. The second, less rapidly falling portion of the curve largely represents metabolic destruction of the factor. It is from this second exponential that the biologic half-life is determined. It is a common error, among practical transfusion therapists to confuse half disappearance time with biologic half-life. The former will, of course, be much faster than the latter and will depend upon the speed and amount of clotting factor lost from the circulation before equilibrium is established. It is much more important to know the half disappearance time of a clotting factor than its biologic half-life when one is faced with the necessity for maintaining its level in the circulation above any given value. Other important considerations are as follows: equilibrium between intravascular and extravascular pools may take place at different rates with different proteins; when increased capillary permeability is present (as may occur more frequently than is appreciated after nonautologous plasma transfusions),[49] the volume of distribution of a plasma protein may become proportionately larger; the rate of synthesis of an individual protein may affect the actual amount shifted before equilibrium is established; disappearance rates found in normal subjects may not necessarily apply to patients with reduced clotting factor concentrations, and vice versa; the amount of clotting factor injected may influence its disappearance rate, larger amounts sometimes disappearing more slowly than smaller quantities;[60] the disappearance rate

may be increased by inflammation and other concomitant conditions. In determining net recovery the initial dilution caused by plasma expansion must be taken into account. The calculated net recovery of the infused protein at zero time after injection must be found by extrapolation of the first exponential of the disappearance curve; this may require that multiple specimens be taken in the immediate post infusion period; a single specimen taken at 15 minutes may not be adequate since complete mixing in the intravascular compartment may require a longer time.

Fibrinogen: The disappearance rate of fibrinogen has been more extensively studied than that of any other clotting factor. Gitlin[40] and Ingram[50] measured its concentration after infusion of a concentrate in patients with afibrinogenemia. The former used immunochemical and the latter gravimetric methods to measure fibrinogen. Others[24, 43, 67] have used radioactive labeled fibrinogen in normal and fibrinogenopenic patients. The half disappearance time was 12 to 36 hours; the biologic half-life was four to six days; and the total body pool appeared to be two to five times the size of the intravascular compartment. It appears from these studies that there is no significant difference in the disappearance rate of fibrinogen in normal and congenital afibrinogenemic patients.

Prothrombin: Reports concerning the disappearance rate of prothrombin appear to be completely irreconcilable. Van Creveld[95] stated that no change could be found in the prothrombin concentration one hour after transfusion of 500 ml. of fresh blood to one of his patients, a 12-year-old boy suffering from a congenital deficiency of the factor. Borchgrevink, *et al.*,[19] in a careful study of a 38-year-old woman with a similar deficiency found a half disappearance time of 24 hours, a biologic half-life between two and one-half and three days, and an apparent total body pool of about one and one-half times the intravascular compartment. Quick, *et al.*,[75] found little effect after transfusion of blood or plasma stored at 4 C., yet a satisfactory response followed the use of fresh frozen plasma in their patients with congenital prothrombin deficiency. Improvement in the Quick prothrombin test persisted for longer than 48 hours. Biggs and Douglas[15] in a study of a 26-year-old man with idiopathic acquired prothrombin deficiency found that the prothrombin concentration was elevated to 23 per cent of normal following transfusion of 860 ml. of fresh citrated plasma. Four hours later the concentration was at approximately the same level but by eight hours it had returned to its pretransfusion value of 11 per cent. A second transfusion of 500 ml. of stored citrated plasma produced a similar rise in the prothrombin concentration, but the effect was almost totally gone in four hours. It is possible that anticoagulants were present in all cases except that reported by Borchgrevink. The gradually decreasing response after repeated transfusions in Quick's case would suggest this complication. Yet it is difficult to explain the difference in effect of fresh frozen and ordinary blood bank plasma which he showed. A quantitative difference in prothrombin in the two types of plasma seems unlikely, since the prothrombin in blood stored at 4 C. appears to be quite stable.

Factor V: Information in the literature regarding the disappearance rate of factor V is also scanty and conflicting. Frank, *et al.*,[35] gave fresh whole blood transfusions on three occasions to a 29-year-old woman with severe congenital deficiency of factor V. On the basis of the Quick prothrombin test they concluded that the effect lasted only four to 12 hours. Alexander and Goldstein[7] produced a net rise of 15 per cent in factor V following transfusion of 1,000 ml. of fresh whole blood to a 17-year-old girl with a similar deficiency. Approximately half the activity had disappeared in 12 hours

and most of it was gone in 24 hours. Field and Ware[33] transfused fresh plasma (amount unstated) to a 17-year-old girl with total deficiency of this factor and noted 5 per cent elevation which fell to zero in six days.

Factor VII: Hitzig and Zollinger,[44] in studies on an infant with severe factor VII deficiency, found a biologic half-life of 115 to 135 minutes. Frick[36] reported a biologic half-life of 330 to 360 minutes following auto-transfusion of 1,000 ml. of plasma in four subjects with Warfarin induced factor VII deficiency; but when his data are recalculated using the net increase in factor VII over the baseline value (rather than the total concentration) in plotting the disappearance curve, the half-life is found to be 145 to 165 minutes; furthermore, since he measured a complex of both factors VII and X the true half-life shown in his experiments was undoubtedly shorter. This criticism regarding methods of measurement also applies to the biologic half-life of 50 to 55 hours found by Didisheim, *et al.*,[26] following the administration of concentrated extract of clotting factors to a patient with cirrhosis of the liver. Loeliger, *et al.*,[65, 66] reported a biologic half-life of 275 to 300 minutes following plasma transfusion in three patients with congenital factor VII deficiency, and 300 to 340 minutes after transfusion of 450 ml. of whole blood in four patients with coumarin induced factor VII deficiency. However, their results are open to question because too few points on the disappearance curves were determined and the concentrations of factor VII on which many of the crucial points were based, were too low for accurate measurement. Data in the literature are insufficient to determine half disappearance rate or total body pool of factor VII following transfusion of plasma in the human subject. Hoag, *et al.*,[46, 47] using a concentrated extract in patients with severe congenital and acquired (Warfarin and liver disease) factor VII deficiency found a half disappearance time of 20 to 60 minutes, a biologic half-life of 80 to 120 minutes, and an apparent total body pool of two to four times the intravascular pool. In patients with cirrhosis of the liver, with baseline factor VII concentrations of 5 to 40 per cent, the half-life was 90 to 120 minutes. In normal subjects and patients with mild and moderate congenital deficiency of factor VII they found a biologic half-life of 210 to 240 minutes.

Factor VIII: Many investigators apparently have considered the disappearance curve of factor VIII following plasma transfusion to be a single rather than a double exponential function.[13, 20, 21, 28] The half-life found in these experiments ranged between four to 11 hours. Pool and Robinson[74] found a double exponential curve with a biologic half-life of 10.2 hours. Their data would indicate a half disappearance time of seven hours and an apparent total body pool of one and one-half times the intravascular pool. Surgenor, *et al.*,[93] found a biologic half-life of ten to 15 hours after the injection of a factor VIII concentrate in patients with severe congenital deficiencies. They mention one patient, however, in whom a steady exponential fall, with a half-life of 2.7 hours, was observed. All of the above experiments were performed on patients with severe congenital deficiency of factor VIII, and the concentration of the factor was determined by biologic assay methods. Adelson, *et al.*,[4] used isotope tagging combined with immunologic separation to study the survival of small amounts of factor VIII transfused into normal humans. The donors were given single injections of S^{35} labeled methionine. Twenty-four hours later they were phlebotomized and the blood was immediately transfused into normal subjects. Factor VIII was separated from the plasma by specific antibodies. The factor VIII antibody was prepared by immunizing rabbits with a partially purified factor VIII prepa-

ration and absorbing the antiserum with factor VIII deficient plasma. Precipitins obtained by mixing the antiserum with the radioactive plasma samples were counted in a liquid scintillation counter. A double exponential disappearance curve with a biologic half-life of 62 hours was obtained. Although this is remarkably longer than that found by all previous observers it can probably be explained on the basis of the ability of the immunologic method to accurately measure small quantities of factor VIII. Since it required 24 hours for equilibration to occur it is probable that previous investigators have detected only the first exponential of the disappearance curve. It is important to note, however, that despite the relatively long biologic half-life, the half disappearance time of seven and one-half hours shown by Adelson's data is in close agreement with the findings of others. His data indicate an apparent total body pool of approximately twice the size of the intravascular pool.

Factor IX: Applying similar methods to a study of factor IX Adelson, *et al.*,[4] found a half disappearance time of 36 hours, a biologic half-life of 9.2 days, and an apparent total body pool approximately three times the size of the intravascular pool. There are no other reliable studies in the literature regarding the disappearance rate of factor IX, although, since the original description of the severe congenital deficiency of this factor, the prolonged effect of transfusion on clotting time and other coagulation parameters has been noted.[5, 98]

Factor X: Graham[41] studied the disappearance rate of factor X in a patient with severe congenital deficiency of this factor. Although there was considerable scatter of the points, the disappearance rate of factor X, studied over a period of five days, appeared to be a single exponential function with a half-life of approximately 47 hours.*

* We have recalculated Graham's data after subtracting the patient's baseline concentration of 3 per cent of factor X from all points given.

Duckert[29] stated that a few experiments carried out on a patient with congenital factor X deficiency and on patients treated with Marcoumar gave a half-life of 40 to 50 hours. It is possible that the results reported by these authors, like the earlier ones on factor VIII, may reflect only the first exponential of a double exponential disappearance curve. In the absence of further data, it is impossible to state what the apparent total body pool of factor X may be in relation to the intravascular pool.

PTA Factor: Because of the lack of a suitable assay method no data is available regarding the disappearance rate of PTA. On the basis of the degree of improvement in various clotting tests it would appear that in most cases it lies somewhere between that of factor VIII and factor IX.[22, 85] However, there are several reports of patients, particularly those with mild deficiencies, whose coagulation defect has either not been corrected by plasma transfusions or has shown temporary correction only after massive amounts.[64, 76, 77, 84] Although it was distinctly stated that no anticoagulants were present, it was possible, at a later date, to demonstrate an anti-PTA in one of them[52] by the use of more definitive technics.

Minimum Hemostatic Levels

Since the occurrence of clinical bleeding is greatly influenced by the nature and degree of stress placed upon the hemostatic mechanism, it is difficult to define minimum hemostatic levels for platelets and clotting factors, except in relative terms.

Platelets: The "critical level" of the platelet count has been accepted as 50,000 per cu. ml. for many years, yet it is well known that a poor correlation exists between the platelet level and the occurrence of purpura. It is probable that most patients will bleed spontaneously with levels below 10,000 per cu. ml., but will not bleed with levels above 100,000 per cu. ml. However, some patients undergoing major

surgery will bleed with levels of 150,000 per cu. ml.

Fibrinogen: In acquired surgical and obstetric hypofibrinogenic syndromes the critical level for bleeding is in the region of 100 mg. per 100 ml.[68] Although hemorrhage is a prominent feature in congenital afibrinogenemia, coagulable blood is not essential for at least some degree of hemostasis, since patients with this disease may experience long periods of freedom from hemorrhage.[8] However, they may bleed severely following trauma or ulceration. In such episodes it is usually necessary to maintain the fibrinogen concentration between 50 mg. and 100 mg. per 100 ml. until hemostasis is established.

Prothrombin: Most of the cases of congenital prothrombin deficiency have had levels close to 10 per cent of normal. Borchgrevink, *et al.*,[19] have speculated that lower levels may be incompatible with life. There was a surprising lack of clinical response in their patient to transfusions of plasma in sufficient amounts to raise the prothrombin levels to 19 per cent. On the other hand, Quick's[75] and Sacks and Raccuglias'[87] cases appear to have had satisfactory responses from amounts of plasma which would probably not raise the prothrombin concentration above this level. Furthermore, the prothrombin concentration in many patients on long term anticoagulant therapy, who show no evidences of abnormal bleeding, is frequently maintained in the region of 20 per cent of normal.

Factor V: Fantl[31] has stated that the critical level for factor V is probably in the region of 30 per cent of normal. However, most patients with severe congenital factor V deficiency have had levels of less than 5 per cent. Whenever it has been used, fresh whole blood transfusions have promptly controlled bleeding (*35.92*). It is doubtful whether the factor V concentration could have been raised to a level of greater than 10 per cent in these patients. On the other hand Kingsley,[55] in a study of 37 members of one family found factor V levels of less than 5 per cent in the three who had a definite bleeding tendency; some bleeding was encountered among 19 members with the heterozygous state of deficiency when the factor V levels were below 40 per cent.

Factor VII: Voss and Waaler[96] found no tendency to bleed in patients with hereditary partial factor VII deficiency with levels over 25 per cent of normal. It is known that patients receiving long term oral anticoagulant therapy do not necessarily bleed at surgery with levels of prothrombin and of factors VII, IX and X in this region.[11, 38] However, Hoag, *et al.*,[46] have noted one patient with levels of 26 to 35 per cent of factor VII who has had numerous bleeding episodes. Shulman[89] has repeatedly controlled epistaxis in a child with severe congenital factor VII deficiency by achieving levels in the region of 25 per cent with transfusions of 250 ml. of plasma.

Factor VIII: Patients with mild deficiencies of factor VIII with levels as high as 35 per cent of normal have less bleeding than the severe cases whose levels are always below 5 per cent. However, even the mild cases almost always bleed after tooth extraction, tonsillectomy, and major surgical operations.[6] Graham, *et al.*,[42] postulated a minimum hemostatic level of 20 per cent. Langdell, *et al.*,[60] noted that hemorrhages in hemophilic patients are often controlled with an amount of plasma which probably would not raise the factor VIII level above 5 per cent. Blombäck and Nilsson[18] using a concentrated factor VIII preparation encountered bleeding after surgical operation when the factor VIII level was below 10 to 15 per cent. Biggs and Macfarlane[16] recommended levels of 30 to 40 per cent, which can usually be achieved only with concentrated preparations, to stop bleeding from major wounds or operative sites. They

found that at a level of 25 per cent nearly all patients bled more than normally following tooth extractions, but bleeding was much less than would be expected to occur in an untreated severely affected patient.

Factor IX: The minimum hemostatic level for factor IX is probably comparable to that for factor VIII. The majority of mildly affected patients have similar symptoms and degrees of specific clotting factor deficiency,[6] and the amount of blood or plasma required to produce hemostasis appears to be equal to that required in factor VIII deficiency of the same degree of severity.[23] The factor IX concentration in most patients receiving oral anticoagulant therapy is usually not far below the "therapeutic range" of 15 to 25 per cent activity indicated by the Quick prothrombin test.[91] Bachmann, *et al.*,[11] found factor IX levels between 2 per cent and 10 per cent, while other factors were within the therapeutic range, in one-fourth of all patients who bled while on Marcoumar therapy.

Factor X: There is little information regarding the minimum hemostatic level for factor X. Graham[41] was able to prevent bleeding after tooth extraction in a severely deficient patient by a single transfusion of 1,055 ml. of plasma, which produced an immediate elevation of the factor X concentration to 23 per cent. The level of factor X in the heterozygous state of congenital deficiency, in which apparently little abnormal bleeding had occurred, has been reported to be between 21 per cent and 80 per cent.[29, 41, 86] We have encountered a moderate bleeding tendency, particularly after major surgery, in a patient with 40 per cent of factor X. Since the level of this factor is reduced to a degree comparable to that of prothrombin and factors VII and IX after long term oral anticoagulant therapy[48] it is probable that its usual requirements for hemostasis are also similar. Bachman, *et al.*,[11] found factor X levels of 3 to 10 per cent, when the Quick prothrombin times were still in the "therapeutic range" of 15 to 25 per cent, in half of all patients who bled while on Marcoumar therapy.

PTA Factor: Rapaport[81] using a new assay method for PTA has found levels between 6 per cent and 20 per cent in patients who bled following major surgery. Levels of 30 to 60 per cent were observed in the minor form of the disease (heterozygous state), where slight abnormal bleeding was only occasionally found. PTA did not appear to be influenced by oral anticoagulant therapy.

Multifactor Deficiencies: The question may fairly be raised whether, in complex multifactor deficiencies, it is necessary to maintain all clotting factors above a given level in order to assure hemostasis, or whether some factors are more important than others. In patients with decompensated hepatocellular disease there may be major deficiencies of platelets, prothrombin, fibrinogen, and factors V, VII, IX and X.[34, 79] Since it was found by Finkbiner, *et al.*,[34] that ordinary bank blood, stored 11 to 18 days, proved less effective than fresh blood in controlling surgical bleeding in such patients, it must be assumed that either factor V, or platelets, or both required replacement. However, it cannot be determined which of the other factors was the more important. On the other hand, it has been repeatedly demonstrated, in the complex platelet and clotting factor deficiencies which occur as complications in certain surgical and obstetrical patients, that elevation of the fibrinogen concentration alone, by the use of concentrated extracts, may produce a satisfactory hemostatic response.[68] In this situation elevation of the factor V concentration, which is sometimes markedly reduced, would appear to be unimportant. We have observed a patient on long term anticoagulant therapy, whose Quick prothrombin test was 27 per

cent, prothrombin 16 per cent, factor VII 26 per cent, and factor X 9 per cent, who bled severely following tooth extraction. Administration of a concentrated extract[46] raised his Quick prothrombin test to 65 per cent, prothrombin to 21 per cent, factor VII to 89 per cent, and factor X to 14 per cent and resulted in immediate and permanent cessation of bleeding. It is difficult to say whether the marked increase in factor VII concentration, the minimal increase in prothrombin and factor X, a combination of these effects, or their summation as indicated by the Quick test was responsible for the production of hemostasis. It will obviously require much further investigation with purified, concentrated clotting factor extracts to clarify many of the problems involved in attempting to define minimum hemostatic levels in those conditions having multifactor deficiencies.

Clinical Considerations

In addition to the well known hazards involved in the transfusion of blood or plasma, such as the transmission of viral hepatitis, there are special circumstances which complicate the problem when dealing with hemorrhagic disorders. The use of non-autologous plasma may cause increased capillary permeability, with loss of protein into the tissues, and, in some cases, actual contraction of plasma volume,[49] which could result in less effective elevation of clotting factor levels in the blood following transfusion. Where very large amounts of plasma are required hypervolemia with cardiac embarrassment and an increased bleeding tendency may ensue. Furthermore, unless type specific single units, or plasma from large pools is used, acute hemolytic anemia due to high isoagglutinin titers may be produced. Unless carefully drawn fresh whole blood is used in combating anemia and shock there may be a considerable reduction in the platelet count.[56] Furthermore, a peculiar failure of hemostatic response, not clearly due to thrombocytopenia, clotting factor deficiency, or the development of anticoagulants, may occur in some patients following massive whole blood or plasma transfusions. In some of the congenital clotting factor deficiencies repeated plasma transfusions may lead, over a period of time, to the development of specific anticoagulants. Continued use of plasma in such patients may only serve to accentuate the clotting abnormality.[14, 37, 63]

It should be obvious from the foregoing that normal blood plasma is not an ideal medication for the treatment of hemorrhagic disorders. If it had been designed specifically for this purpose it would be considered a poor product. It contains a polyglot combination of items, all of which must be given, when frequently only one is required. Few of its components are present in sufficient concentration to allow for really effective treatment of many severe bleeding episodes. It must be collected and stored with the utmost care to avoid deterioration of several of its vital components. In the amounts frequently required its cost is prohibitive. It is vitally necessary that specific clotting factor concentrates, similar to the presently available fibrinogen and to other products, which have so far been used only experimentally,[17, 18, 26, 27, 46, 47, 53, 61, 93] be developed for general use. However, for the present, plasma is our only remedy for a wide variety of hemorrhagic disorders and it behooves us to take full advantage of its limited potential by devoting our attention to its proper storage and use.

The following very general concluding remarks regarding transfusion therapy of hemorrhagic disorders seem justified. For the treatment of thrombocytopathia and idiopathic or secondary thrombocytopenic purpura fresh whole blood or fresh platelet rich plasma should be employed. A sufficient amount should be given to raise the

platelet count of the recipient to at least 100,000 per cu. ml. This may prove to be impossible in ITP or when the patient has previously received numerous transfusions. Plasma for the correction of factor V or VIII deficiency may be either fresh, freshly lyophilized and stored for periods up to several years, or freshly frozen and stored for variable periods at —20 C. Storage for longer than three weeks at —20 C. is not advisable unless specific assays on the plasma in a given blood bank indicate that its methods permit longer retention; if they do not the technics of collection and storage should be investigated. Under favorable circumstances storage for at least one year is allowable. For the treatment of deficiencies of all other clotting factors, plasma drawn from blood kept at 4 C. for 21 days and then stored at —20 C. for several months thereafter appears satisfactory. In the initial infusion a sufficient amount of plasma should be given to raise the deficient clotting factor concentration in the recipient's blood to 10 to 50 per cent of normal, depending on a variety of circumstances. The higher levels may prove impossible to achieve or maintain without the aid of concentrated clotting factor preparations. As an initial infusion, 1,000 ml. of normal plasma to an average sized adult is usually required to produce minimum hemostatic levels. The amount and frequency of subsequent transfusions will depend mainly upon the status of the patient and upon the *half disappearance rate* of the particular clotting factor or factors which require replacement.

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Optimal Conditions for Storage of Fresh Frozen Plasma *

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Small aliquots of plasma in 3.8 per cent trisodium citrate were stored at −15, −30 and −50 C. Lengthening of the recalcification time after 24 hours followed by progressive shortening for 10-14 days was observed at −15 and −50 C. At −30 C. no significant changes occurred until two months had elapsed. Assay of clotting factors VII and X, VIII and IX revealed essentially that the activity of each rose gradually over a span of 12-14 days after an initial decline at −15 C. and −50 C. At −30 C. factors VIII and IX were stable for at least two months. Factor V level declined gradually at −15 C. and −50 C. but no significant fall was observed at −30 C. The increase in the activity of factor VIII observed during storage at −15 and −50 C. was eliminated by prior adsorption with Celite which is believed to remove an activation product of Hageman factor and Plasma Thromboplastin Antecedent. There seemed to be somewhat greater instability of plasma stored in untreated glass containers than in cellulose nitrate or in siliconized glass. Storage temperatures below −40 C. and above −25 C. appear to be unfavorable for plasma coagulation factors.

THIS PAPER presents experimental data collected in an attempt to delineate the optimal physical conditions for the storage of fresh frozen plasma. Particular attention has been paid to the influence of the storage temperature, the nature of the storage vessel and the method of preparing the plasma before freezing. The levels of the principal coagulation factors, expressed in terms of a percentage of the activity of a normal plasma which was used throughout, were determined initially and at intervals during the course of the various experiments. The results of these experiments throw light on the stability of the plasma factors under various physical circumstances, and allow certain definite conclusions to be drawn regarding the most suitable temperature for the storage of fresh frozen plasma.

Materials and Methods

Collection of Blood: Blood was collected through a silicone-coated metal needle into the recipient container, one volume of 3.8 per cent trisodium citrate for nine volumes of blood collected. Trisodium citrate was used exclusively as the anticoagulant in this study.

Citrated Plasma: Plasma was obtained by centrifugation of blood collected as described above for 15 minutes, at 3,000 rpm. in a refrigerated centrifuge at 4 C. The plasma was then transferred to a second tube using a siliconed Pasteur pipette, and spun for a further 15 minutes. This plasma was "platelet-free."

Glassware: Standard 75 x 10 mm. Pyrex glass test tubes were used in the experiments. All pipettes used in handling plasma together with some of the collecting and storage vessels, were silicone-coated, a water soluble silicone preparation (Siliclad, Clay-Adams, Inc., New York) being used exclusively. In the initial experiments, new test tubes were used and all glassware was cleaned according to the following procedure:

Glassware was washed with soap and water, rinsed and then steeped for 24 hours in chromic-sulphuric acid. After a tap

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water rinse it was boiled for 30 minutes in 15 per cent sodium carbonate, rinsed and immersed for 24 hours in 10 per cent potassium hydroxide. It was then washed 20 times in tap water, five times in distilled water and dried in an oven.

Veronal Buffer: Veronal buffer of *p*H 7.3 and ionic strength 0.15 prepared from the formula: Sodium diethylbarbitone 11.745g., sodium chloride 14.43g., 0.2N HCl, 15 ml., distilled water to two litres.

Recalcification Time: A system containing 0.2 ml. test plasma 0.2 ml. veronal buffer, and 0.2 ml. M/40 $CaCl_2$ was used.

Prothrombin: The method of Hjort, *et al.*,[3] was employed.

Prothrombin Time: Prothrombin time was measured by Quick's technic using brain thromboplastin.

Barium Sulfate Adsorption: 100 mg. $BaSO_4$ powder was added to each ml. of plasma. The mixture was stirred for ten to 15 minutes at room temperature with a siliconed glass rod, then the powder removed by centrifugation.

Celite Adsorption: To the plasma was added 25 per cent of its volume of Celite powder, a diatomaceous SiO_2 supplied by the Great Lakes Carbon Corporation, New York. The mixture was tilted for ten minutes at room temperature, then the powder removed by centrifugation.

Assay of Factor V: Factor V was estimated by Wolf's modification of Stefanini's one stage method.[7]

Assay of Factor VII Complex: A method similar to the Stuart-Prower factor assay of Bachmann, *et al.*,[1] was employed with the difference that brain thromboplastin replaced the stypven-lecithin in the incubation mixture.

Assay of Factor VIII (Antihemophilic Factor, AHF): A modification of the thromboplastin generation technic was employed. A calibration curve was constructed by preparing percentage dilutions of a fresh normal plasma in hemophilic plasma and determining the thromboplastin-forming capacity of the mixture by the two-stage method. The mean value of the clotting times of normal substrate plasma, after addition of subsamples of the incubation mixture at six, seven, eight and nine minutes, was used to read the factor VIII level off the calibration curve. This value was not subject to significant variation.

Factor IX (PTC, Christmas Factor): Factor IX was assayed by a variation of the above technic. The procedure was exactly the same as for factor VIII assay, except that the hemophilic plasma was replaced by intact plasma from a severe case of Christmas disease. Unless the factor IX deficient plasma was freshly collected it was "exhausted" with regard to Hageman factor–PTA activity by the technic of Waaler[6] before use in the assay. The factor IX assay seems very susceptible to the influence of contact-induced activation. Satisfactory factor V and VIII levels are maintained by this technic.[6] Test plasmas stored under uncontrolled conditions before assay may also be treated in this way as the method does not influence the stability of factor IX.

Refrigeration Temperatures: Constant temperatures of −15, −20, −30, −40, and −50 C. were maintained in several deep freezes. These temperatures were constantly checked by automatic recording devices reflecting very minor degrees of change, and were supervised by refrigeration engineers.

Results

1. Influence of Storage Temperature on Plasma Recalcification Time: The recalcification time was selected as a simple and convenient means for detecting overall changes in the clotting mechanism. Preliminary experiments were carried out on aliquots of normal plasma stored in lusteroid tubes at −15, −30, −40, and −50 C. The recalcification time of the plasma was determined initially and on an aliquot of

plasma from each of the several temperatures at three day intervals for 27 days. No change in the −30 C. specimens was observed, but in those stored at −15, −20, and −50 C. a progressive shortening of the recalcification time was observed, reaching its peak at 10-14 days in the case of the −50 C. sample and somewhat later in the case of the −15 and −20 C. samples. The −40 C. samples gave somewhat variable results, but shortening of the recalcification time was also noted. It was decided to study in detail the behavior of plasma samples stored in various containers at −15, −30, and −50 C.

Plasma from two donors (HA and MGR) was obtained "platelet-free" from blood collected in plastic (cellulose nitrate) containers through a siliconized needle, thus eliminating as far as possible contact with a surface known to induce contact activation. Aliquots of each plasma were stored in plastic tubes at −15, −30 and −50 C., and tested at the intervals shown in Figure 1.

The results show that at −50 C. a lengthening of the recalcification time occurs after 24 hours storage to be followed by a progressive shortening which reaches a minimum value at ten to 14 days. Thereafter, the times lengthen again but do not return to their initial level, remaining distinctly

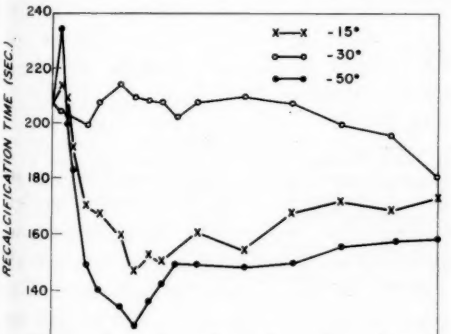

FIG. 1. The effect of storage (in lusteroid containers) at three different temperatures on the recalcification time of fresh normal plasmas.

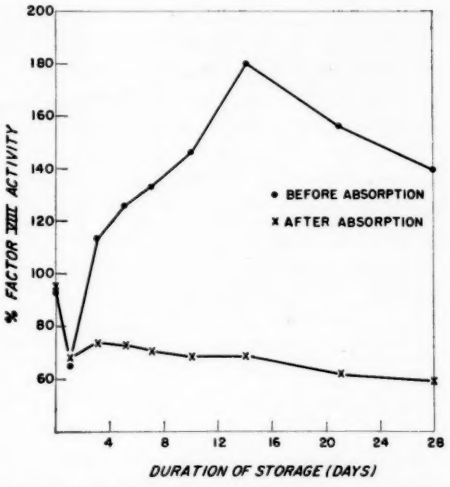

FIG. 2. The behavior of the apparent level of factor VIII (AHF) activity in plasma stored at −50 C. before and after treatment with Celite.

shorter in each case. Similar changes take place at −15 C. but here the initial increase in the recalcification time was not so pronounced. Storage at −30 C. results in no significant changes until at least two months have elapsed, when similar, but much more gradual changes begin.

2. Influence of Temperature on Levels of Clotting Factors: Blood was collected through siliconized needles into trisodium citrate contained either in "lusteroid" cellulose nitrate plastic tubes or in siliconized glass tubes. "Platelet-free" plasma was prepared as before and distributed in plastic tubes in 1 ml. aliquots which were stored at −15, −30 and −50 C. Portions could thus be thawed and tested at intervals. Each sample was discarded after testing. The factors assayed were V, VII "complex," *i.e.*, factor VII plus factor X (Stuart-Prower factor), factor VIII (AHF) and factor IX (PTC, Christmas factor). Prothrombin was estimated immediately after collection, again at the peak of plasma activity, and finally after the peak had been passed. Six normal plasmas were examined in this way over a period of 28 days.

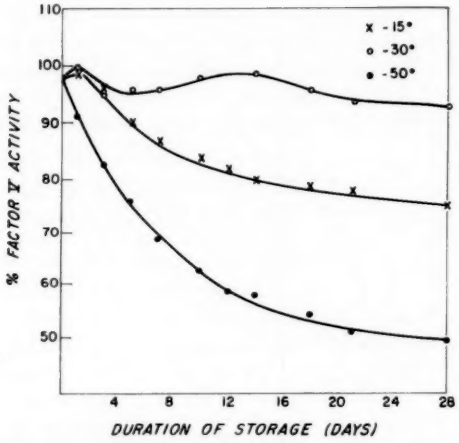

FIG. 3. Showing the behavior of factor V on storage of normal plasma in lusteroid containers at three different temperatures.

At −30 C. no changes in the apparent levels of factors VIII and IX occurred over a period of 28 days. At −50 C., the value for factor VIII showed an initial fall averaging about 30 per cent of the mean initial activity of the plasmas tested. This fall was followed by an apparent rise progressing to a peak at 12-14 days in which the observed level appeared to have increased considerably above its original value. This was followed by a decline in activity but even after 28 days the measurable level was still substantially higher than the original value. Parallel studies of factor IX activity showed essentially similar results with the exception that the initial fall in activity was not observed. Peak activity was reached at 12-14 days as in the case of factor VIII. The behavior of factor VIII at −50 C. is shown in Figure 2 upper curve. At −15 C. similar changes in the behavior of factor VIII and IX activity were noted but these were of lesser magnitude.

Figure 3 shows the results for factor V. At −50 C. the factor V level declined gradually until at 28 days it had fallen to about half its initial value. At −15 C., some fall in activity was seen, but is of much smaller magnitude. At −30 C., no significant change in factor V was observed.

3. An Attempt to Correct the Apparent Increase in Factor VIII and IX Activity: The above changes cannot represent the

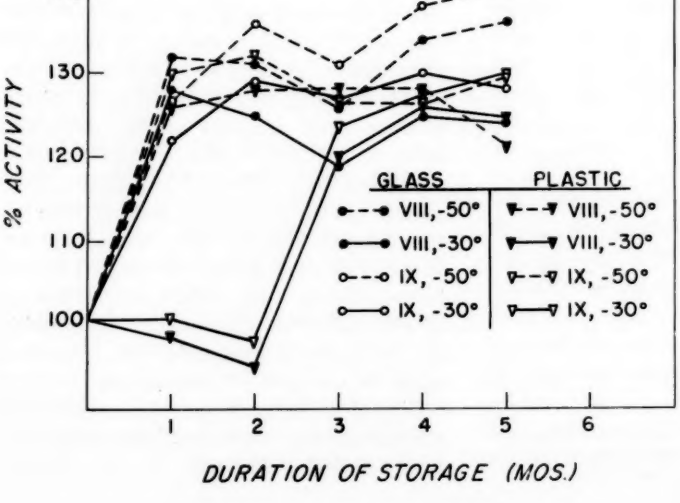

FIG. 4. The behavior of the apparent level of factor VIII (AHF) activity of plasma stored in both untreated glass and plastic containers at two different temperatures.

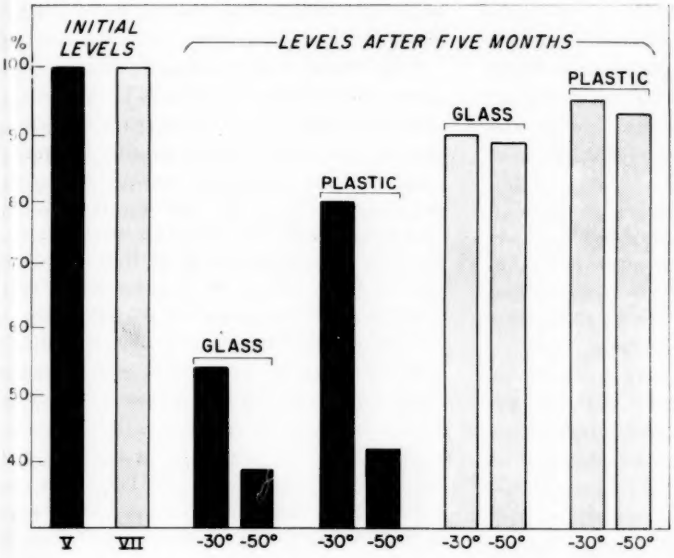

FIG. 5. The behavior of factors V and VII over a period of five months in plasmas stored in untreated glass and plastic containers at two different temperatures.

true behavior of factors VIII and IX, especially as factor VIII showed an initial fall in its level. They resemble the changes which can be induced in a plasma by exposure to glass which results in Hageman factor activation. Some process similar to contact activation must be responsible for the accelerated thromboplastin formation observed here.

Since Hageman-like activity was thought to be responsible for the activation just described as occurring at −50 C. and −15 C. the same series of six plasmas was treated as before, and factor VIII assayed at intervals. Part of each sample, however, was adsorbed with Celite powder (vide Methods) and the powder removed before being assayed, whilst the other portion was assayed without such treatment. Waaler[4] has shown that such treatment causes maximal activation of Hageman factor with formation of an activation product in association with Plasma Thromboplastin Antecedent (PTA), and that about 90 per cent of this material is adsorbed on to the powder.[5] If the increase in activity were due to Hageman factor activation, it should be largely eliminated by adsorbing the plasma with Celite.

Figure 2, lower curve, shows the results. Much of the additional activity was removed by Celite adsorption but the observed level of factor VIII remained a little higher than the 24 hour level and subsequently there was not much change. The figures obtained after Celite adsorption are believed to represent truer values for factor VIII.

4. The Influence of the Type of Container: All results described so far were for plasmas stored in plastic containers. Four "platelet-free" plasmas were next stored in aliquots in plain, un-siliconed, glass tubes at −30 C. and −50 C. Recalcification time and assays of Factor V, VII "complex," VIII, and IX were carried out as before and at intervals of one month over a period of five months. The overall results are shown in figures 4 and 5.

At −50 C. the results follow the same pattern as for plasma stored at this temperature in plastic, but the peak activity de-

veloped a little earlier (seven to ten days). Treatment with Celite reduced the apparent factor VIII and IX activity to a figure which was more likely correct. The behavior of factors V, VII "complex," VIII, and IX were thus similar to those described for storage in plastic.

Contrary to the findings in plasmas stored in plastic some changes were observed in plasmas stored in glass at −30 C. These show a gradually developing increase in activity (as judged by the recalcification time) reaching its peak level at 21 days to 28 days. Factors V and VIII showed a gradual but slower decline and the VII complex and factor IX were unchanged. Thus there seemed to be somewhat greater instability of plasma stored in glass rather than in plastic (Fig. 4).

Four plasmas were also tested in the same manner after storage in siliconed glass tubes. These behaved in an identical way to those stored in plastic tubes. Figure 4 also shows the results of storing aliquots of normal plasma in plastic containers for a period of five months at −30 C. It will be observed that there is no change in activity over the first two months, but at three months activation occurs similar to that observed in glass containers or in plastic at −50 C. Thus it would appear that even at −30 C. changes do occur in plasma stored in plastic, but that these changes are negligible over the first two months.

Discussion

The results of these experiments indicate that both the nature of the storage vessel and the storage temperature are of importance in the preservation of fresh frozen plasma.

At −15 C., and again at −50 C., changes are induced in plasma which resemble the activation resulting from glass contact. Concomitant with these changes there seems to be a partial consumption of at least two important coagulation factors, factor V and factor VIII.

The initial fall in factor VIII activity observed consistently at −50 C. and to a smaller extent at −15 C. suggests the possibilities that either this globulin is undergoing denaturation and thereby losing its potency or else some reaction is induced as a result of exposure to the low temperature which involves the quantitative utilization of factor VIII. Such a reaction would be most likely to take place on thawing.

The subsequent marked increase in the apparent level of factor VIII activity is the result of the activation of some factors or factor. That most of this activity can be removed by Celite adsorption suggests that the activated agent might be Hageman factor and that what is removed on the powder might be the "activation product" formed by the interaction of Hageman factor and Plasma Thromboplastin Antecedent (PTA). Certainly, the fall in activity cannot be accounted for by the removal of factor VIII on Celite since Waaler has shown that factor VIII is adsorbable to only a very slight extent on this material.[5] Moreover, the fact that a parallel increase in the apparent activity of factor IX takes place indicates that this is a non-specific activation phenomenon. Gaston,[2] working independently, found similar changes occurring at temperatures below −50 C. Our results confirm his observations and demonstrate clearly the occurrence of activation on freezing plasma at these low temperatures.

After Celite adsorption of the activated plasma the factor VIII level seems to remain at a constant low level. That the falling off of factor VIII is not a continuous process is somewhat surprising, and suggests that some definite changes take place as a result of storage at too high or too low a temperature for 24 hours in which a fixed quantity of factor VIII is consumed. The gradual decline of factor V at these temperatures might reflect that it, too, is involved in such a process.

In any event, whatever the explanation of these interesting phenomena, which we have called "freezing activation," several points emerge which are of considerable practical importance. First, the unreliability of unmodified assay technics for factors VIII and IX and the failure to recognize the occurrence of activation phenomena may lead to the belief that a plasma has a high content of these factors when it in fact does not. Secondly it is apparent that storage of fresh frozen plasma at temperatures around −50 C., or above −20 C. may lead to decay of at least two major clotting factors. Thirdly the use of glass containers as storage vessels seems to hasten the process and allows it to begin even at −30 C.

Conclusions

1. The optimum storage temperature is −30 C.

2. At −30 C. plasma is completely stable for at least two months, and maintains a fairly high content of factor VIII for about four months.

3. Storage at temperatures below −40 C. and above −25 C. is to be avoided.

4. The results suggest that there may be a theoretical advantage in the use of plastic storage vessels rather than glass, but until data from *in vivo* transfusion experiments are available it is not certain how great this advantage might be. No changes in existing methods of collection are justified until this point is elucidated.

Summary

Fresh frozen plasma was stored at three temperatures −15, −30, and −50 C. for periods up to five months. Its stability with regard to clotting factors was determined by assays of the principal factors at intervals. It was found that changes resulting in shortening of recalcification time and decrease in content of antihemophilic activity and factor V activity occurred at −50 C. and in a lesser degree at −15 C., irrespective of the nature of the storage vessel. At −30 C. these changes were delayed for two months, up to that time all factors remaining stable provided that storage was in plastic or siliconized vessels. In glass, changes were observed at all three temperatures. The nature of these changes is tentatively discussed. It is felt that −30 C. is the most suitable temperature for storage of frozen plasma.

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The Influence of Low Temperature-Storage on the Heparin Tolerance of Human Plasma*

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Plasma stored at −50 C. or lower develops a resistance to heparin as demonstrated by progressive shortening of the thrombin time. Adsorption with Celite powder or barium sulfate before or after freezing eliminates this effect. Plasma kept at −30 C. did not develop resistance to heparin after seven days.

RECENT STUDIES[1, 2] have indicated that the exposure of citrated plasma to temperatures of −50 C. or lower for periods of 24 hours or longer results in certain changes which accelerate the formation of thromboplastin in the plasma, and thus increase the apparent level of activity of those plasma components directly concerned in thromboplastin formation, *i.e.*, factors VIII (AHF) and IX (Christmas factor, PTC). This activation has certain points in common with that resulting from glass contact.[1] In investigating this phenomenon, which we have called "freezing activation," studies were carried out to determine whether or not the exposure of plasma to temperatures of −50 C. or below influence other aspects of the clotting mechanism, one of which was the resistance of plasma to varying amounts of heparin. The results indicate that significant changes in heparin tolerance are induced by exposure to such temperatures.

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Materials and Methods

Heparin - Thrombin Tolerance Tests: Using a siliconed needle and syringe, nine parts of blood were collected by venipuncture into "Lusteroid" plastic tubes containing one part of 1.34 per cent sodium oxalate. After mixing by inversion (the mouth of the tube being covered by a sheet of "Parafilm"—Marathon Corp., Menasha, Wis.), the blood was centrifuged for 15 minutes at 3,000 rpm. in an International Refrigerated Centrifuge, model PR-2, of radius 5.5 inches, at 4 C. The plasma was then transferred to a second plastic centrifuge tube, using a siliconed Pasteur pipette and respun as before for a further 15 minutes.

The platelet poor plasma was then transferred to a third plastic tube, which was stoppered and kept in melting ice during the test. Aliquots of plasma could be transferred to further small plastic tubes and frozen at any desired temperatures.

A calibration curve was constructed using the fresh plasma by adding to it aliquots of dilutions of sodium heparin solution ranging from 0 to 2.0 units per milliliter. The incubation mixture comprised:

0.2 ml. test plasma
0.1 ml. of heparin solution (or distilled water)
0.2 ml. of either thrombin, 2.5 or 5μ/ml. or 1.0 M calcium chloride.

The thrombin was rapidly blown into the incubation mixture as a stopwatch was started.

Because of the very long values of the recalcification time obtained when higher concentrations of heparin were used, thrombin times were determined in most of these tests. Recalcification times were used to check the validity of the results with the thrombin time method.

For each dilution of heparin three readings were obtained and the mean of these readings was taken as the final result. A curve was then constructed in which the clotting time was plotted as ordinate and concentration of heparin as abscissa.

Heparin: "Liquaemin" brand of sodium heparin containing 1,000 units per ml. in aqueous solution was used to prepare the dilutions (Organon).

Thrombin: Bovine powdered thrombin was used to prepare the thrombin solutions. (Upjohn Co.).

Glassware: All pipettes used in the transfer of plasma were siliconed. Test tubes were used once only, and all new tubes used were cleaned by immersion in chromic sulphuric acid for 24 hours, followed by immersion in 15 per cent potassium hydroxide for a further 24 hours. They were then washed 20 times in tap water, three times in distilled water and dried in an oven.

Silicone: "Siliclad" brand of water soluble silicone (Clay-Adams, Inc., N. Y.) in 5 per cent dilution in warm distilled water was used to coat glass pipettes and syringes. For needles used in venipuncture a 50 per cent solution was used and the needles were steeped for one hour.

Results

Fresh plasma was obtained as above from seven normal individuals and a calibration curve constructed. Aliquots of the plasmas were then frozen in plastic tubes and stored at −50 C. Individual samples could thus be thawed and tested at intervals. After testing the remains of each sample were discarded.

Samples were frozen rapidly to −79 C. using a solid CO_2-acetone freezing mixture, then maintained at −50 C. in a closed deep freeze for 15 min., 30 min., two and six hours. Further samples were stored for one to 14 days at −50 C. At each time interval a sample was thawed in a water bath at 37 C., and the calibration curve repeated on the thawed sample using heparin and thrombin from precisely the same batches used to prepare the control calibration curve. As an additional control this

TABLE 1. *Exposure of a Normal Plasma to a Temperature of −50 C. for up to Six Hours Causes No Alteration in its Response to Heparin. Exposure for 24 Hours and Longer Causes a Sharp Decrease in the Response of Plasma to Heparin.*

Conc. of heparin in mixture (μ/ml.)	Thrombin Times of HA Plasma Stored at −50 C.							
	Fresh plasma control	After 15 min.	After 6 hrs.	Fresh plasma control	After 24 hrs.	After 3 days	After 5 days	After 14 days
	Thrombin 5μ/ml.			Thrombin 2.5μ/ml.				
	SEC.	SEC.	SEC.	SEC.	SEC.	SEC.	SEC.	SEC.
0.00	12.5	12.2	12.4	17.0	16.0	15.8	11.5	11.0
0.025	—	—	—	17.5	—	—	—	—
0.05	14.8	15.0	15.0	24.0	16.5	16.5	14.0	13.4
0.10	18.0	18.0	17.8	38.6	18.0	17.4	15.5	15.5
0.15	23.2	—	—	57.4	—	—	19.0	15.8
0.20	26.5	27.0	25.5	60.5	25.2	26.4	22.1	17.4
0.25	31.5	32.6	33.0	—	—	—	—	—
0.30	39.3	40.2	38.6	—	—	—	—	—
0.40	—	—	—	112.8	61.5	68.0	50.2	37.4
0.50	73.5	70.0	70.2	—	—	—	—	—
0.60	85.2	83.4	84.5	—	—	—	—	—
0.70	104.5	107.1	105.0	—	—	—	—	—

TABLE 2. *Storage of Plasma at −30 C. for from One to Seven Days Has No Influence on the Response of the Plasma to Heparin.*

Conc. of heparin in mixture (μ/ml.)	Thrombin Times of HA Plasma Stored at −30 C. Fresh Plasma Control 1st day of experiment	7th day of experiment	After 24 hrs.	After 7 days
	SEC.	SEC.	SEC.	SEC.
0.00	12.0	12.5	12.00	12.2
0.10	17.5	18.0	18.8	17.5
0.30	38.5	40.0	41.0	40.2
0.50	70.2	73.5	74.2	71.5
0.70	105.6	106.4	104.5	102.5
1.00	185.2	182.4	186.4	180.0

curve was repeated using fresh plasma from the same individual at each time interval during the 14 day period of observation.

The results presented in Table 1 show that storage up to six hours does not in any way influence the response of the plasma to heparin, thus demonstrating that the simple physical process of freezing and thawing alone does not produce any change. Storage for 24 hours and longer, however, produces a profound decrease in the response to heparin. The results shown in Table 1 are those obtained with one plasma (HA), but these are typical of the findings with all seven plasmas tested.

In contrast to these results, when the same plasmas were stored at −30 C., no significant alteration in heparin tolerance was found even after seven days (Table 2). Thus exposure to a lower temperature seemed to be instrumental in producing this remarkable effect. Fresh specimens of the same plasmas, tested each day in parallel with the frozen samples, gave closely reproducible results.

Heparin was next added to a sample of plasma from one of us (HA) to give a concentration of 2μ/ml. The thrombin clotting time was then determined using thrombin of 5μ/ml. strength. The plasma was then frozen in two aliquots, one at −30 C. and the other at −50 C. After seven days each sample was tested in the same manner. It was found that the thrombin clotting time of the −50 C. sample had shortened considerably, so that the apparent heparin content had fallen from 2μ/ml. to about 0.7μ/ml. The sample stored at −30 C. showed no significant change. As an additional control, a portion of the aqueous solution of heparin used in heparinizing the plasma initially was also stored for one week at −50 C. After thawing, it showed no loss in potency.

It has been observed that plasma frozen at temperatures of −50 C. or below for periods in excess of 24 hours develops a greater reactivity in the thromboplastin generation test whether the two stage procedure or the one stage procedure based on the cephalin time is used.[1, 2] There is some evidence to suggest a relationship between this freezing activation and Hageman factor activity.[1, 2] The possibility of a relationship between this type of activation and the decreased response to heparin observed over the low temperature range was therefore investigated.

For this purpose, nine parts of blood were collected into one part of 3.8 per cent trisodium citrate in a "Lusteroid" tube, using a siliconed needle and syringe. The plasma was next treated with 25 per cent of its volume of Celite powder, agitated gently for one minute at room temperature and the powder removed by centrifugation. It was then stored, along with samples not

so treated, at −50 C. in aliquots of 1.0 ml. which were thawed and tested at intervals as before. Figure 1 illustrates the results. Not only was there no increase in heparin resistance, but over a period of seven days the response of the plasma to heparin, especially to the higher concentrations, became more marked. The untreated samples, however, showed the decreased response as expected. One of these samples, stored for seven days, showed a sharply increased response to heparin following adsorption with Celite powder after thawing (Fig. 1). Thus Celite adsorption either before freezing or after thawing directly influenced the response of the plasma to heparin.

Adsorption with barium sulfate of samples of plasma (as in the thromboplastin generation test) before freezing them to −50 C. also prevented the development of an increased heparin resistance over a period of seven days.

Discussion

The phenomenon of a decrease in the response of normal plasmas to increasing concentrations of heparin as measured by the thrombin clotting time induced apparently by exposure to low temperature is of much theoretical interest. Care was taken in all the experiments to make absolutely certain that there was no contact between the plasmas tested and any foreign surface known to induce contact activation. Thus at the time of freezing the plasmas were "platelet-free" and not activated. In spite of this, changes took place after 24 hours at −50 C., but not at −30 C. Treatment of the plasma before freezing with Celite powder or barium sulfate prevented the change in the response of the plasma to heparin.

The interpretation of these findings is very difficult. Waaler[3] has shown that the only coagulation factors adsorbed to a sig-

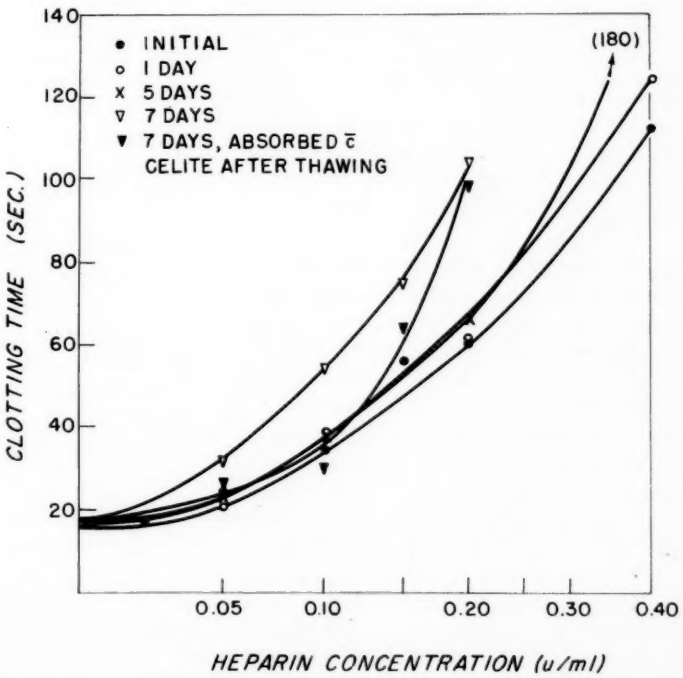

FIG. 1. The influence of Celite adsorption on heparin resistance of plasma stored at −50 C. Plasmas, unless otherwise indicated, were adsorbed before freezing.

nificant extent onto Celite are the Hageman factor and plasma thromboplastin antecedent (PTA). However, it is difficult to attempt to find any connection between this fact and the behavior of plasma with regard to heparin after storage at −50 C., since one is reluctant to attribute this altered behavior to changes in factors which are primarily concerned with the formation of thromboplastin. Yet the fact remains that both barium sulfate adsorption and Celite adsorption will prevent the appearance of a changed response to heparin, and Celite adsorption of a plasma exhibiting the change results in its reversal. The implications of these findings are thus obscure.

The principal action of heparin is dependent upon its formation of a complex with an albumin component of plasma variously called Albumin X, heparin co-factor, etc. Another possibility is that the prolonged storage of plasma at −50 C. results in changes in the co-factor which might in turn lead to interference with the action of heparin. However, in the absence of experimental findings to support this contention, this must remain purely speculative. Clearly further experimental work is indicated to elucidate the nature of this problem.

Summary

The heparin resistance of plasma can be influenced by freezing the plasma at temperatures at −50 C. or below for 24 hours or longer. This treatment causes a substantial increase in heparin resistance both when measured by the recalcification time method or by the thrombin time method. This increase in resistance can be prevented by adsorption of the plasma with Celite before or after freezing, and also by barium sulfate adsorption. The implications of this observation are briefly discussed.

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Transfusions in the Treatment of Hemorrhagic Diseases*

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The transfusion of fresh or fresh frozen plasma serves as a specific means to correct in part and temporarily the coagulation defect in hemophilia A, hemophilia B, hereditary hypoprothrombinemia vera, and congenital deficiencies of labile, stable and Stuart-Prower factors (factors V, VII and X). The effect of a transfusion can usually be measured by simple tests such as the prothrombin time and the prothrombin consumption time. To correct thrombocytopenia by transfusion, platelet-rich plasma should be given. Whole blood should only be used when the bleeding is massive and continuous or if the hemoglobin is very low. Transfusion of either whole blood or plasma into patients who have congenital or acquired anticoagulants in the blood, such as heparin or antithromboplastinogen (AHG or factor VIII), is ineffective and is likely to accentuate particularly the latter condition.

THE READY availability of blood and particularly of fresh frozen plasma has had a great impact on the management of the hemorrhagic diseases. Transfusions even when given empirically are often lifesaving. With a better understanding of the mechanism of hemostasis and of the coagulation of blood, therapy can now be put on a more rational basis. In doing this, complex and conflicting theories of coagulation need not be considered. The main requirement is a simple practical concept of hemostasis and a few reliable methods suitable for routine use with which the coagulation defect can be localized and measured quantitatively. With this equipment, it is possible to decide when a transfusion is indicated, what kind is to be given, and often to determine the effect quantitatively.

Hemorrhage can be divided into two major types: spontaneous and traumatic. The first is closely associated with capillary dysfunction. The normal capillary allows the diffusion only of solutes having relatively small molecular weights. It retains plasma proteins and the cellular elements. Under pathological conditions this selective diffusion may be drastically altered—even red cells may escape from the capillaries by diapedesis—giving rise to purpuric bleeding. This is manifested by showers of petechiae, ecchymoses, bleeding from mucous membranes particularly of the nose, gums, mouth and sometimes of the vaginal and gastro-intestinal tracts. The true nature of capillary bleeding is poorly understood and is difficult to study because of the paucity of specific laboratory tests. Platelets appear to be closely associated with capillary function.

Traumatic bleeding involves vessels larger than the capillaries. Usually bleeding is from an arteriole or small artery. This type of hemorrhage may be subdivided into two classes. In the first, coagulation of the blood is normal whereas in the second, a clotting defect can be demonstrated. The classical example of the first class is telangiectasia (Rendu-Osler disease). The telangiectasia is a dilated uncontractile vessel which, when traumatized, will bleed unabated despite the normal clotting of the blood. Stanching can be effected only by compression of the vessel. This clearly illustrates that the coagulation of blood is

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ineffective when the vessel remains uncontractile, and supports the hypothesis that the first essential step in hemostasis is a reflex contraction of the injured vessel and that this is followed by a series of events of which the formation of the fibrin clot is a terminal step and probably the beginning of the reparative process. It seems certain that only when the initial vascular response to injury is normal is it possible for the fibrin clot to be utilized effectively in permanent hemostasis.

This intimate interrelationship and interdependence between the vascular and the clotting reactions make it difficult to separate these two facets of the hemostatic mechanism for the purpose of evaluating the part each contributes to stanching. The vascular factors are particularly difficult to determine because of the lack of satisfactory methods. The bleeding time and the tourniquet test offer some help but have serious limitations.

Since various reliable methods have been developed to determine the primary clotting factors, hemostasis is evaluated mainly in terms of coagulation. Greatest clinical success in treating bleeding with transfusions has been in those diseases in which the coagulation defect is established and the correction accruing from transfusion of normal blood or plasma can be quantitatively measured.

During the past ten years the effect of transfusion in various hemorrhagic diseases has been studied, especially for estimating changes in the clotting factors immediately at the completion of the transfusion. In this study blood was drawn immediately before and after the transfusion using silicone-coated equipment and promptly transferred to thick-walled silicone-coated test tubes which were kept in an ice bath. Blood was drawn through the needle used for the transfusion. The methods for determining the various clotting factors have been described in detail.[4, 6] Only methods which are relatively simple and suitable for routine study were selected. The average age of the fresh frozen plasma transfused was two months (range three days to eight and one-half months). In presenting the results, each bleeding disease is treated separately in order to explain adequately the experimental approach and to interpret the results.

Fresh frozen plaza was prepared from blood drawn into double plastic bags containing 75 ml. of anticoagulant acid citrate dextrose solution U.S.P. formula A. Centrifugation and separation were performed at 4 C. Final storage was in glass containers which were slant-frozen and kept in a deep freeze at −25 C. Fresh blood was collected in glass bottles into the same anticoagulant and was considered to be "stored blood" after 24 hours of storage.

Stored pooled plasma was prepared from outdated bank blood. The processing of this plasma includes radiation with ultraviolet light and "polishing" by passage through a press filter with cellulose pads.

Platelet-rich plasma was prepared by differential centrifugation of fresh blood collected into a plastic bag or siliconized bottle. When larger quantities of platelets were used the method of concentration described by Tobin and Friedman[7] was employed.

Hemophilia

Basic Defect: A marked lack of thromboplastinogen (factor VIII) or antihemophilic globulin in the plasma.

Diagnosis: True hemophilia can be established unequivocally by the following triad: (1) a history of bleeding of the traumatic type beginning in early childhood, (2) a sex-linked recessive pattern of heredity, and (3) a markedly low concentration of thromboplastinogen.

Methods for Evaluating the Effect of Transfusion—Clotting Time: Irrespective of how long the clotting time is, it should become normal after a transfusion. Since it is apt to be normal in mild hemophilia, the

TABLE 1. *Assay for Thromboplastinogen*

Prothrombin consumption time	Volume of plasma* in ml.				
sec.	0.005	0.01	0.05	0.1	1.0**
	Per cent of normal				
9	4.0	2.0	0.4	0.2	0.02
10	8.0	4.0	0.8	0.4	0.04
11	14.0	7.0	1.4	0.7	0.07
12	18.0	9.0	1.8	0.9	0.09
13	22.0	11.0	2.2	1.1	0.11
14	28.0	14.0	2.8	1.4	0.14
15	32.0	16.0	3.2	1.6	0.16
16	36.0	18.0	3.6	1.8	0.18
17	40.0	20.0	4.0	2.0	0.20
20	54.0	27.0	5.4	2.7	0.27
25	80.0	40.0	8.0	4.0	0.40
30	100.0	50.0	10.0	5.0	0.50

* Added to a mixture of 1 ml. of native plasma from a severe hemophiliac, 0.1 ml. of hemolysate and .03 ml. of aged serum.

** Determination made directly on the patient's plasma.

test is of no value in estimating the effect of transfusion in such patients.

Prothrombin Consumption Test: Although the test is not a specific measure of thromboplastinogen, a close correlation between the concentration of this clotting factor and the prothrombin concentration of the serum is found. After a transfusion, the prothrombin concentration is almost always increased. The test is of little or no value in mild hemophilia when the consumption of prothrombin is already normal before the transfusion.

Hemolysate Prothrombin Consumption Time: In this procedure, an extract of erythrocytes (hemolysate) is added to the blood before it is clotted. In both true hemophilia and hemophilia B, the prothrombin time of the resulting serum is low except in the very mild cases and is increased after a plasma transfusion.

Hemolysate + Serum Prothrombin Consumption Time: Normal aged serum and hemolysate are added to the blood before it is clotted. The prothrombin time of the resulting serum may be regarded as a fairly

TABLE 2. *The Effect of Transfusing a Small Volume of Fresh Plasma in a Severe Hemophiliac*

		Transfusion of 10 ml. of fresh normal plasma		
		Before	After 5 minutes	After 60 minutes
Clotting time	min.	60	17	
Prothrombin consumption time	sec.	8	8	8
Hemolysate + serum time	sec.	8	22	14
Thromboplastinogen concentration:				
Observed	%	<0.01	0.3	0.14
Calculated*	%		0.3	

* On the basis of mixing 10 ml. of normal plasma with 3,000 ml. of hemophilic plasma.

TABLE 3. *The Effect of Transfusion of Fresh Frozen Plasma on Two Severe Hemophilic Boys*

		Subject 1 (7 yrs. old)				Subject 2 (8 yrs. old)							
		January 1959		January 1960		May 1957		January 1960			June 1960		
		Before	After	Before	After	Before	After	24 hrs. Later	Before	After	24 hrs. Later	Before	After	24 hrs. Later
Clotting time	min.	37	7	31	8.5	58	9.5	13	100	8.5	21	45	6	12
Prothrombin consumption time	sec.	7.5	11	8	13	8.5	13	12	8	17.5	11.5	9	24	13
Hemolysate + serum time	sec.	8	66	9	33	8	61	71	9	52	35	9	47	43
Thromboplastinogen assay	%			0.05	3				<0.05	7	0.6	<0.05	5.5	2

reliable quantitative measure of the concentration of thromboplastinogen, provided it is not over 0.5 per cent of normal. When the concentration exceeds 0.5 per cent, the assay method for thromboplastinogen must be employed using plasma from a severe hemophiliac as the testing medium.

Thromboplastinogen Assay: For this test, native plasma from a severe hemophiliac serves as the assay medium. It is obtained by collecting the blood with silicone-coated equipment and centrifuging at high speed to remove the platelets. Such plasma can be preserved for several months at −20 C. in silicone-coated tubes. To 1 ml. of the hemophilic plasma, 0.03 ml. of aged normal serum and 0.1 ml. of hemolysate are added and the mixture transferred to a glass test tube and put in a water bath at 37 C. In this mixture all the known factors needed for the generation of thromboplastin are present except thromboplastinogen. In the complete absence of the latter, the serum prothrombin time is eight seconds. When 0.001 ml. of normal plasma is added to this mixture, the prothrombin consumption time is increased to 12 seconds. With increasing amounts of normal plasma, the prothrombin consumption time increases in a straight line ratio. On this basis, a table has been constructed for calculating the concentration of thromboplastinogen (Table 1).

The accuracy of the assay method was tested by the following experiments: By means of silicone-coated equipment, 10 ml. of native plasma from a normal subject was obtained. This was immediately injected intravenously into a severe hemophiliac. The results are recorded in Table 2.

Results Obtained from Transfusions: The results presented in Tables 3 and 4 are typical of the findings repeatedly obtained in a group of 20 true hemophiliacs ranging from mild to severe. As seen from the data, the clotting defect remains fairly constant in a hemophiliac and likewise his

TABLE 4. *The Effect of Transfusion of Fresh Frozen Plasma in a Moderately Severe Hemophilic Adult*

		March 1959			November 1959	
		Before	After 250 ml.	After 500 ml.	Before	After 250 ml.
Clotting time	min.	16	5	5	15	6.5
Prothrombin consumption time	sec.	10	16.5	16	9.5	12
Hemolysate + serum time	sec.	31	99	112	23	114
Thromboplastinogen assay	%	0.5	6	8	0.3	2.3

response to plasma transfusions often shows little variation over a period of years. The clotting time becomes normal immediately after the plasma is given; the basic prothrombin consumption time and the hemolysate-serum tests show significant improvement. The most reliable means for estimating quantitatively the effectiveness of a transfusion is the thromboplastinogen assay. A significant increase is obtained. Since the concentration of thromboplastinogen varies in normal individuals, not all plasmas have the same potency. This accounts in part for the difference in response as measured by the increase of thromboplastinogen. The rate of utilization of this factor is variable. In Subject 2, only 0.6 per cent of activity remained for 24 hours on one occasion, while several months earlier 2 per cent was still present on the second day; yet, the concentration immediately after the transfusions was about the same on both occasions.

Although no attempt is made in this paper to correlate the laboratory findings with the clinical response, it should be stated that no direct relationship between the clinical effectiveness and the quantitative increase of thromboplastinogen after transfusions has been observed. Often the improvement sets in after the thromboplastinogen has dropped. The rapid metabolic turnover of this clotting factor makes it appear unlikely that its activity is restricted merely to being a static plasma constituent kept in reserve for the generation of intrinsic thromboplastin when a vessel becomes injured. Such observations as the rapid reabsorption of blood from an extensive intramuscular hemorrhage which is frequently observed to follow promptly a plasma transfusion suggest that the beneficial effect may in part come from an improvement of vascular function.

Hemophilia B
(PTC Deficiency or Christmas Disease)

Basic Defect: A marked deficiency of a plasma factor, different from thromboplastinogen, which results in a poor production of thromboplastin as measured by the thromboplastin generation test or the prothrombin consumption time. The factor is designated PTC (plasma thromboplastin component), factor IX or Christmas factor.

Diagnosis: As in true hemophilia, the diagnosis is based on a triad:

1. A history of bleeding of the traumatic type from early childhood.
2. A sex-linked recessive pattern of heredity.
3. A markedly low concentration of PTC.

The latter can only be estimated indirectly in serum since it is present in an inactive state in plasma.

Methods for Evaluating the Effect of Transfusion: As in hemophilia, a patient with hemophilia B will have following a transfusion of fresh frozen plasma a normal clotting time and the basic prothrombin consumption time and the hemolysate test will be or approach normal. The hemoly-

TABLE 5. *The Effect of Transfusion of Fresh Frozen Plasma in Hemophilia B*

		Subject 1						Subject 2	
		June 1958				June 1959*		May 1959	
		Before	After 250 ml.	After 500 ml.	After 24 hrs.	Before	After	Before	Af er
Clotting time,	min.	35	10	10	15	30	8	22	5
Prothrombin consumption time:									
Basic	sec.	12	16	16.5	11.5	10.5	13	9.5	11
With hemolysate	sec.	19	17	29.5	25	12.5	47	18	31
With hemolysate + serum	sec.	46	92	102	27	130	86	89	90

* Reconstituted lyophilized fresh plasma was given. Patient had a marked allergic reaction but bleeding from a cut on the tongue promptly stopped.

sate + serum test is always normal because normal serum supplies PTC, therefore this test is of no value for the purpose of evaluating the effectiveness of transfusions in hemophilia B.

Results Obtained from Transfusions: Fewer studies were made on hemophilia B patients than on true hemophiliacs because only six patients reside in the Milwaukee area and these rarely required hospitalization and transfusions.

The effect of fresh frozen plasma transfusions is recorded in Table 5. The clotting time is promptly reduced to normal but is rather insensitive as compared with the basic prothrombin consumption test and the hemolysate test. The latter two show that when a patient is given a second unit of 250 ml. of plasma further correction of the blood is obtained. Unfortunately, no reliable method is available for assaying PTC such as has been developed for thromboplastinogen. The problem is complicated because PTC has to be activated before it can be measured and it is difficult to know whether a deficiency of PTC activity is due to an actual decrease of the factor or to defective activation.

TABLE 6. *The Effect of Tranfusion of Fresh Frozen Plasma in a Girl with Severe Hereditary Hypoprothrombinemia*

		Transfusion of 250 ml. Before	After
Clotting time	min.	>60	7
Prothrombin time:			
Basic	sec.	73	18
With added serum	sec.	60	18
With added labile factor	sec.	51	16
Total prothrombin time	sec.	40	16

Hereditary Hypoprothrombinemia Vera

Basic Defect: A marked deficiency of prothrombin with no decrease of labile factor nor a significant alteration in the concentration of stable factor.

Diagnosis: The disease is recognized by a prolonged one-stage prothrombin time that is not corrected by the addition of normal aged serum or by fresh rabbit plasma adsorbed with $Ca_3(PO_4)_2$. The diagnosis is further confirmed by a low value of prothrombin obtained by means of the Iowa two-stage method modified by Ware and Seegers.

Since the defect is transmitted as an autosomal recessive, it is rarely possible to establish heredity, but the carrier state sometimes can be detected by a slightly prolonged one-stage prothrombin time.

Methods for Evaluating the Effect of Transfusion: The one-stage prothrombin time is the most suitable and convenient method for determining the effect of a transfusion. It is both simple and reliable.

Results Obtained from Transfusions: The studies are based on three subjects with this disease. Two are brothers who have been repeatedly studied. Their prothrom-

bin times remain in the range of 19 to 26 seconds. The prothrombin as measured by the two-stage method is 10 per cent of normal. They are relatively mild bleeders. The third is a girl aged nine years who has required a plasma transfusion every five weeks since birth. Her prothrombin time is always over 40 seconds and several times has been found to be over 65 seconds.

There has been only one opportunity to study her response to a plasma transfusion. The results are recorded in Table 6. By means of 250 ml. of fresh frozen plasma, the prothrombin time was reduced from 73 to 18 seconds. From her total prothrombin time which is the serum prothrombin time of the plasma depleted of platelets, it can be calculated that her prothrombin concentration before transfusion was about 2.5 per cent and increased to 10 per cent after the plasma was given.

Numerous studies on the effect of transfusions have been carried out on the two brothers with hypoprothrombinemia. It was found early that stored blood or stored pooled plasma brought about little correction of the prothrombin time nor did it appear to benefit the patient clinically. In contrast, fresh frozen plasma greatly lowered the prothrombin time and the effect remained for over 48 hours as seen in Table 7. Interestingly, as the two brothers have become older, the amount of plasma needed to correct the prothrombin time has steadily increased. At the age of ten years, 250 ml. of fresh frozen plasma decreased the prothrombin time to 13.5 seconds. At present 500 ml. reduces it only to 16 seconds.

The girl who has a much more severe hypoprothrombinemia develops with amazing regularity hemorrhage into her thighs five weeks following a plasma transfusion. Several days after a transfusion the swelling from the hemorrhage subsides and though her prothrombin time continues to fall, she has no further bleeding for five weeks. The beneficial effect of the plasma appears to depend on more than merely increasing temporarily the prothrombin level.

Hereditary Labile Factor (Factor V) Deficiency

Three siblings with this disease have been studied, but there has been no opportunity to test their response to a plasma transfusion. The condition is easily diagnosed by a prolonged one-stage prothrombin time which is corrected completely by the addition of labile factor. Rabbit plasma adsorbed with $Ca_3(PO_4)_2$ is a satisfactory means for supplying this factor for the test. The effect of a transfusion is quantitatively determined by the shortening of the prothrombin time. Because labile factor decreases on storage, fresh or fresh frozen plasma is the recommended agent to administer.

TABLE 7. *The Effect of Transfusion on a Subject with Moderate Hypoprothrombinemia*

	Prothrombin time Before sec.	After sec.	
January 21, 1955	20	17	500 ml. stored blood
January 28, 1955	23	23	350 ml. stored pooled plasma
January 29, 1955		22	350 ml. stored pooled plasma
January 30, 1955	22	13.5	250 ml. fresh frozen plasma
		16	24 hours later
		16	48 hours later
		20	72 hours later
January 1956	22	14.5	250 ml. fresh frozen plasma
		15.5	24 hours later
		18.5	48 hours later
		25	72 hours later
April 1957	24	17	250 ml. fresh frozen plasma
		17	24 hours later
		18	48 hours later
April 1959	23	17	250 ml. fresh frozen plasma
		15	Second 250 ml. fresh frozen plasma
May 1960	20	17.5	250 ml. fresh frozen plasma
		16	Second 250 ml. fresh frozen plasma

TABLE 8. *The Effect of Transfusion of Plasma in Stable Factor (Factor VII) Deficiency*

		Before	After	
Patient 1, age 32:				
August 1954				
Clotting time	min.	11.5	10.5	250 ml. banked pooled plasma
Prothrombin time	sec.	46.5	47	
February 1958				
Clotting time	min.	9	6	250 ml. fresh frozen plasma
Prothrombin time	sec.	61	26	
Prothrombin consumption time	sec.	33	19	
February 1960				
Clotting time	min.	9	9	250 ml. fresh frozen plasma
Prothrombin time:				
Basic	sec.	41	25	
With aged serum	sec.	13	12.5	
With added labile factor	sec.	40	24	
Prothrombin consumption time	sec.	28	21	
Patient 2, age 9:				
July 1958				
Clotting time	min.	9	9	250 ml. fresh frozen plasma
Prothrombin time:				
Basic	sec.	70	22	
With added aged serum	sec.	13	12.5	

Hereditary Stable Factor (Factor VII) Deficiency

Basic Defect: A lack of a plasma constituent which is essential for the utilization of tissue thromboplastin in the conversion of prothrombin to thrombin. This factor is variously designated as proconvertin, SPCA (serum prothrombin converting factor) and factor VII.

Diagnosis: The unequivocal diagnosis of stable factor deficiency is difficult, especially its differentiation from the condition designated as Stuart-Prower defect. A detailed discussion of this problem is not warranted in this presentation. For practical purposes the one-stage prothrombin time is adequate. In pure stable factor deficiency, the prolonged prothrombin time is completely corrected by adding aged normal serum to the plasma.

Methods for Evaluating the Effect of Transfusion: The one-stage prothrombin time is the only simple procedure available to determine quantitatively the effect of transfusions.

Results Obtained from Transfusions: Three cases of hereditary stable factor deficiency have been studied but none has been available for repeated and extended investigation. In one patient, the effect of a serum transfusion was determined. The prothrombin time before the transfusion was 38 seconds; it was 20 seconds four hours after the transfusion and 44 seconds 24 hours later.

Fresh plasma or fresh frozen plasma have been found most effective clinically and the effect can be readily measured by the one-stage method. Stored pooled plasma was found ineffective. The studies made on a second patient are particularly interesting, because her condition has been diagnosed, first, as a hypoprothrombinemia,[3] later, as stable factor deficiency,[1] and recently, as Stuart-Prower deficiency.[2] The results of a recent study are presented in Table 8. The

decrease of the prothrombin time is not striking but nevertheless definite. Interestingly, 11 years earlier, Dr. Hagen studied the effect of injecting 250 ml. of plasma into the patient and obtained almost identical values: 40 seconds before and 23 seconds after the transfusion.

The third patient, a girl aged nine years, is a severe bleeder who requires a transfusion every two or three weeks. One study of the effect of a fresh frozen plasma transfusion was made (Table 8). By means of 250 ml. of plasma the prothrombin time was reduced from 70 to 22 seconds. The clinical response was prompt. In less than 24 hours the swelling due to a hemorrhage into the right forearm subsided, but two weeks later she had another hemorrhage.

Hereditary Thrombocytopenia

Basic Defect: A low platelet count due apparently to a congenital inability to produce an adequate number of platelets to maintain the normal level.

Diagnosis: The laboratory tests for establishing the disease are the platelet count and the prothrombin consumption time. The history of bleeding and purpura soon after birth is essential to determine that the disease is congenital.

Methods of Evaluating Effect of Transfusion: The platelet count before and immediately after the transfusion will furnish the essential information concerning the effectiveness of transfusion, but it should be complemented by a prothrombin consumption test before and after the transfusion. For this condition, fresh blood collected in a plastic container or siliconized bottle or platelet-rich plasma prepared from such blood should be given because the platelets remain intact and functionally active.

Results Obtained from Transfusions: Studies have been limited to one boy who has a severe thrombocytopenic purpura since birth. He lost sight in both eyes after intraocular hemorrhages. Even for minor dental work, he requires a platelet-rich plasma transfusion. In Table 9 the effects of such a transfusion are recorded. An increase of the platelet count and of the prothrombin consumption are consistently obtained. This boy has not responded to a splenectomy or to prolonged treatment with cortisone.

Acquired thrombocytopenia is not included in this presentation because transfusions are rarely an important part of the therapy.

Acquired Antithromboplastinogenemia

Basic Defect: The presence of a specific substance in the plasma which promptly inactivates thromboplastinogen.

Diagnosis: The condition can be suspected whenever a prolonged clotting time is not corrected by a fresh frozen plasma transfusion. The presence of the anticoagulant is established by the failure of increasing amounts of fresh normal plasma to correct the prothrombin consumption time when added to the patient's native plasma that has been mixed with hemolysate and normal aged serum.

Methods of Evaluating the Effect of Transfusion: The same tests are employed in testing the effect of transfusion as in classical hemophilia. No improvement will be noted by any of the tests if the condition is severe.

TABLE 9. *The Effect of a Platelet-Rich Plasma Transfusion in a Boy with Congenital Thrombocytopenia*

		Transfusion	
		Before	After
Clotting time	min.	5.5	5
Prothrombin time	sec.	12	12
Prothrombin consumption time:			
Basic	sec.	10	16
Wih hemolysate	sec.	64	128
Platelet count		30,000	60,000
Clot retraction		very slight	good

TABLE 10. *The Effect of Plasma Transfusion in a Hemophiliac Whose Blood Contains the Anticoagulant, Antithromboplastinogen*

					After 250 ml.*
May 1952	Clotting time	min.		60	
	Prothrombin consumption time:				
	Patient's blood	sec.		8.5	
	1 vol. patient's blood + 1 vol. normal blood	sec.		17	
April 1956	Clotting time	min.		50	30
	Prothrombin consumption time	sec.		8.5	8.5
	Hemolysate time	sec.		9	10
May 1957	Hemolysate time	sec.		9	
	With 0.005 ml. fresh normal plasma added	sec.		26	
October 1958	Clotting time	min.		30+	36
	Prothrombin consumption time	sec.		8.5	8.5
	Hemolysate + serum time	sec.		8	11
July 1960	Clotting time	min.		35	
	Prothrombin consumption time	sec.		8	
	Hemolysate + serum time	sec.		8	
	Patient's plasma	1.0	1.00	1.0	
	Normal plasma	0.005	0.05	0.1	
	Hemolysate + serum time, sec.	8.2	8.2	9.0	
	Control:				
	True hemophilic plasma	1.0	1.0		
	Normal plasma	.0	.005		
	Hemolysate + serum time, sec.	8.0	16.0		

* Fresh frozen plasma transfusion.

Results Obtained from Transfusions: Studies have been limited to one hemophiliac who is now 15 years old. When first seen in 1952, his clotting time was 60 minutes and prothrombin consumption 8.5 seconds. At this time no evidence of a circulating anticoagulant was observed. He was seen again in 1956 when he had hematuria. The response to a plasma transfusion was very poor (Table 10). Evidence for a circulating anticoagulant was obtained. A year later, however, evidence of an inhibitor disappeared. In 1958 he was admitted to the hospital because of hemorrhage into his leg and arm and also hematuria. Immediately after entering the hospital he began to have profuse rectal hemorrhage which continued for four days despite almost continuous transfusion of whole blood and plasma. A study of his blood before and after a transfusion showed that he obtained slight benefit and that his titre of antithromboplastinogen was greatly elevated. After he was given cortisone, the rectal bleeding stopped within a day. No studies were made to determine the response to transfusion.

A recent study showed that his blood still contains a high titre of the anticoagulant.

Congenital Hyperheparinemia

Basic Defect: A high concentration of heparin in the plasma.

Diagnosis: A prolonged clotting time and a prolonged thrombin time which becomes normal when either protamine sulfate or toluidine blue is added to the plasma *in vitro.*

Methods for Evaluating the Effect of Transfusion: The clotting time and the thrombin time before and after the trans-

fusion are the most reliable means to determine the effect.

Results Obtained from Transfusions: The unique opportunity to study a patient with congenital hyperheparinemia at various times over a period of years has furnished valuable data. Since these results have been published previously,[5] a short summary need only be given. The heparin titre was not reduced by the intravenous injection of either toluidine blue or protamine sulfate. The clotting time as well as the thrombin time was significantly shortened by administering cortisone and this was further reduced by transfusions of fresh frozen plasma. Significantly, when fresh frozen plasma was given without cortisone, it had no demonstrable effect (Table 11). Unfortunately, these observations have only been made once. There has been no opportunity to repeat these studies. The beneficial effect of cortisone could not have been coincidental because as soon as the drug was withdrawn the heparin titre began to rise and returned to its original high concentration.

Discussion

The two clearly recognized objectives for transfusions in a bleeding disease are: (1) to correct anemia, and (2) to normalize the coagulation dysfunction. In achieving the latter it is very probable that often hemostasis is improved by effects on the vascular system which are not measurable by any clotting test now available. In meeting these objectives, it is important to know what kind of blood should be given.

Fresh blood from a normal donor should theoretically be the most satisfactory because it corrects anemia and supplies the various essential clotting factors. Practically, however, whole blood is rarely indicated unless the patient's hemoglobin is alarmingly low with the hemorrhage not under control. In whole blood one half of the volume consists of cells which the bleeder usually does not need and which may be disadvantageous to him. Most of the bleeding in a disease like hemophilia is internal; consequently, the blood is eventually reabsorbed and the iron salvaged. Since the organism cannot eliminate iron, repeated transfusions can result in harmful accumulation of this agent. It is also to be emphasized that when many transfusions of whole blood are given, reactions are apt to develop even though the red cells are carefully typed and cross matched.

Fresh normal plasma is the most effective agent for treating hemorrhagic diseases. Almost as effective is fresh frozen plasma which is prepared by freezing the plasma immediately after it is obtained by centrifuging and storing it at −20 C. Under these conditions, the various clotting factors are well preserved. Since all the known clotting agents except platelets are in the plasma, one volume of plasma is equivalent to two volumes of whole blood. By obtaining the plasma from one donor, the danger of hepatitis is no greater than with a whole

TABLE 11. *The Effect of Cortisone and Fresh Frozen Plasma on the Heparin Titre of the Blood of a Patient with Congenital Hyperheparinemia*

		Clotting time min.	Thrombin time sec.	Prothrombin time sec.
July 27, 1956	Before treatment	75	14.5*	27
July 31, 1956	After cortisone for 3 days	25	8	
	After 250 ml. of plasma	15	6.5	
May 14, 1958	Before transfusion	**	16.5	19
	After 250 ml. of plasma		16	19

* Control on normal plasma, 3 seconds.
** No clot within 48 hours.

blood transfusion. The recipient should be given plasma of the correct type in regard to A, B and O.

The advantages of fresh frozen plasma are: (1) it is easy and economical to prepare, (2) it supplies all the known clotting factors present in circulating blood except platelets, (3) it contains no detectable amounts of denatured proteins, consequently, it rarely causes reactions, and (4) it can be administered fairly rapidly. A unit of 250 ml. can be given in less than 30 minutes whereas it may require several hours for a similar volume of whole blood.

Two conditions in which a fresh frozen plasma transfusion fails to bring about any beneficial effect are congenital hyperheparinemia and acquired antithromboplastinogenemia. In the latter condition it actually accentuates the disease. In both, cortisone has been found effective. It is not surprising that this drug counteracts antithromboplastinogenemia since it is likely that the cause is immunological. It should be emphasized that the use of cortisone should be limited to meeting emergencies arising in these two diseases. There is no reliable clinical or laboratory evidence that cortisone is of any benefit in other hemorrhagic states except acquired thrombocytopenic purpura.

In the bleeding diseases characterized by a measurable clotting defect such as hemophilia, and hypoprothrombinemia, the effect of a plasma transfusion can be determined by laboratory tests, and the results agree fairly well with the clinical improvement. In diseases such as pseudohemophilia A (von Willebrand's disease), it is frequently impossible to establish any measurable abnormality. The bleeding time may often be in the normal range. Nevertheless, fresh plasma transfusions are given especially before an operation. The results have been uniformly good, but since such patients without treatment may not have bleeding even after a major surgical operation, the evaluation of the benefit of transfusion needs caution.

Summary

Transfusion of blood and of plasma plays an important part in the treatment and management of the hemorrhagic diseases. The main objectives are the correction of the anemia and the temporary normalization of the coagulation mechanism. It is probable that with the correction of the latter, the vascular response is also improved but this cannot be reliably measured by laboratory tests. The effect of transfusions can be estimated in most bleeding diseases either by the one-stage prothrombin time or the prothrombin consumption time. Since fresh or fresh frozen plasma contains all the known clotting factors except platelets, it is the agent of choice for the treatment of all bleeding states in which a clotting factor is lacking except thrombocytopenia. In two bleeding states in which an anticoagulant is responsible for the defective clotting, namely, hyperheparinemia and antithromboplastinogenemia, neither blood nor plasma is effective, in fact either may accentuate the latter condition. Whole fresh blood is indicated only when the anemia is very severe or the external loss of blood remains out of control.

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Seven Ve^a (Vel) Negative Members in Three Generations of a Family

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A family with seven Vel negative (*VeVe*) members in three generations was found because the propositus had a hemolytic transfusion reaction. She had had four full-term pregnancies and two miscarriages and one previously uneventful transfusion. The anti-Vel antibody in her serum gave both hemolysis and agglutination. Study of the blood groups of the family shows that Ve^a is not in the MNS system. Six of the *VeVe* individuals were tested with anti-Fy^a and all were found to be Fy(a+). Some Ve(a+) bloods were noted to react more strongly with anti-Ve^a than others. The explanation of van Loghem and van der Hart that the differences in the intensity of Ve(a+) reactions is due to subgroups, the stronger termed Ve(a_1) and the weaker Ve(a_2), is accepted by the authors.

THIS REPORT deals with an unusual Caucasoid family that was studied because one member, the propositus, had the rare Vel antibody which will be referred to as anti-Ve^a in this paper. Further studies of members of her family revealed the unique situation of six additional Ve (a—) relatives, *viz.*, two siblings, three maternal aunts and a maternal grandmother.

The propositus, Mrs. L. J. (group A, Rh negative), had four full-term pregnancies, followed by two miscarriages. Following the second pregnancy, the patient was transfused because of postpartum hemorrhage. Apart from a rash on her legs, the transfusion was uneventful. We may assume that anti-Ve^a, if present at this time, would have been detected.

At the second miscarriage, the patient bled profusely and was hospitalized. A blood transfusion was interrupted because of shaking chills and lower lumbar pain followed by anuria. Shortly thereafter she spontaneously passed a fetus of about three months gestation. During the next two days her urinary output was limited to about 100 ml.

The serum of the patient, when tested with 300 donors, hemolyzed and agglutinated these random bloods. Subsequently, on testing members of her family compatible donors were found. The patient then received uneventfully 2 units of Ve (a—) compatible blood of which one unit was Rh positive.

Apparently, the patient was immunized by the first transfusion with presumably Ve (a+) blood and anti-Ve^a which must have been present prior to the second transfusion was not detected in the compatibility test.

When the specimen of the propositus was submitted for study, it was shown that the atypical antibody which gave both hemolysis and agglutination corresponded in specificity to anti-Ve^a first described by Sussman and Miller.[4] Her serum agglutinated all cells in our panel including the rare varieties of —D—/—D—, S^u, O$_h$ (Bombay type), *Tj^{b}Tj^b* (*pp* in newer terminology) and *KK*. It failed to react with the red blood cells of two unrelated Ve (a—) individuals whose sera contained anti-Ve^a. One of these bloods, T.M., was described by Levine, *et al.*, as the second example of anti-Ve^a.[2] The other, M.B., was found in

* Received June 30, 1960; accepted November 14, 1960.

TABLE 1. *Reactions with Random and Selected Bloods*

Red cells tested	Sera T.M.	M.B.	L.J. (propositus)
Random bloods	+	+	+
−D−/−D−	+	+	+
O_h (Bombay)	nt	+	+
S^u	+	+	+
Tj^bTj^b (pp)	+	+	+
KK	+	+	+
T.M. Ve (a−)	0	0	0
M.B. Ve (a−)	0	0	0
L.J. Ve (a−)	0	0	0

The reactions represent hemolysis and/or agglutination.
nt indicates no test.

these laboratories to contain anti-Ve^a.† In both T.M. and M.B., as in this case, the antibody was produced following transfusion.

The findings with the serum and cells of Mrs. L.J. which established the specificity of her antibody as anti-Ve^a are summarized in Table 1 which includes also tests with two group A anti-Ve^a sera mentioned above.

Ve^a and ABO compatible donors among the family of the propositus are shown in Table 2 in parallel tests of her serum with two anti-Ve^a sera. Since the propositus was in group A, Rh negative, the only suitable donor was her brother, III-7. The presence of anti-D was excluded since the serum of the propositus failed to react with Ve (a−), Rh positive donors.

The family tree consisting of four generations is given in Figure 1 and all antigens tested are listed in Table 3. Kidd and Lewis antigens are not recorded because only some of the specimens were tested. Anti-Lu^a was not available to us at the time. The family tree is considerably larger and this report deals with only a portion of those whose specimens were submitted.*

The simplest explanation for Ve^a inheritance is to assume two or more alternative alleles at a single locus. One of these, *Ve*, produces the antigen which reacts with the antibody, anti-Ve^a. The other (s) cannot at present be assigned a superscript, since there are no identifying antibodies. Thus, the assumed allele of Ve^a can be called *Ve*. When an individual is homozygous *VeVe*, his red cells are not agglutinated by anti-Ve^a. The incidence of the three genotypes, as given by Sussman and Miller[4] are:

Ve^aVe^a	Ve (a+) homozygous	.9604
Ve^aVe	Ve (a+) heterozygous	.0392
VeVe	Ve (a−)	.0004

† For this specimen, we are indebted to Margaret Thompson, Blood Bank, St. John's Hospital, Santa Monica, California.

* Additional members of the extended family tree are under investigation by Dr. B. Pirofsky, University of Oregon Medical School.

TABLE 2. *ABO and Rh Types of the Seven Ve(a−) Members of this Family*

	Generation	Blood group	D	C	E	c	L.J.	M.B.	Woud*
W.C., maternal grandmother	I-2	O	+	+	0	+	0	nt	0
M.J., maternal aunt	II-7	O	+	+	0	+	0	0	0
E.T., maternal aunt	II-5	O	+	+	0	0	0	0	0
L.S., maternal aunt	II-13	O	+	+	0	+	0	nt	0
F.D., sister	III-6	A	+	0	+	+	0	0	0
R.R., brother	III-7	A	0	0	0	+	0	0	0
L.J., propositus	III-2	A	0	0	0	+	0	0	0
Random donors							+	+	+

* The content of anti-A and anti-B in this group O serum was neutralized by the addition of group specific substances. This specimen was supplied through the courtesy of van Loghem and van der Hart.

TABLE 3. *Antigens of Family Members Shown in Figure 1*

	Group	D	C	E	c	e	K	k	Fy[a]	S	s	M	N	P	Ve[a]
W C., family:															
♂ -1	O	+	+	0	0		0	+	+	0	+	+	+	+	+
♀ -2	O	+	+	0	+		0	+	+	+	+	+	+	+	0
I-5	O	+	+	0	+		0	+	+	+	+	+	+	0	0
I-7	O	+	+	0	0		0	+	+	0	+	0	+	+	0
I-8	O	+	+	0	+		0	+	+	+	+	+	+	+	+
I-13	O	+	+	0	+		0	+	+	+	+	+	0	0	0
M.(C.), family:															
♀ I-5	O	+	+	0	+		0	+	+	+	+	+	+	0	0
II-9	O	+	+	0	+		0	+	+	0	+	+	+	+	+
III-11	O	0	0	0	+		0	+	+	+	+	+	+	0	+
III-10	A	0	0	0	+		+	+	0	0	+	0	+	+	+
E., family:															
♂ II-6	A	+	+	+	+		0	+	+	0	+	+	0	+	+
♀ II-7	O	+	+	0	0		0	+	+	0	+	0	+	+	0
III-12	A	+	+	+	+		0	+	+	0	+	+	+	+	+w
III-13	A	+	+	+	+		0	+	+	0	+	+	+	+	+w
III-14	A	+	+	0	0		0	+	+	0	+	+	+	+	+w
III-15	A	+	+	+	+		0	+	+	0	+	+	+	+	+w
L.J., propositus and siblings:															
III-2	A	0	0	0	+		0	+	+	0	+	+	+	+	0
III-4	A	+	+	+	+		0	+	+	+	+	+	+	+	+w
III-6	A	+	0	+	+		not tested								0
III-7	A	0	0	0	+		0	+	+	+	+	+	0	+	0
M.C., family:															
♂ III-3	A	+	0	+	+	0	0	+	+	0	+	+	0	0	+
♀ III-4	A	+	+	+	+		0	+	+	+	+	+	+	+	+w
IV-5	A	+	+	+	+		0	+	+	0	+	+	+	+	+
IV-6	A	+	+	+	+		0	+	+	0	+	+	+	+	+
IV-7	A	+	0	+	+	0	0	+	+	0	+	+	+	+	+w
IV-8	A	+	+	+	+		0	+	+	+	+	+	0	+	+w
L.J., propositus–family:															
♂ III-1	O	+	+	0	0		0	+	0	0	+	+	+	0	+
♀ III-2	A	0	0	0	+		0	+	+	0	+	+	+	+	0
IV-1	O	+	+	0	+		0	+	0	0	+	0	+	0	+
IV-2	A	+	+	0	+		0	+	+	0	+	+	+	+	+w
IV-3	A	+	+	0	+		0	+	+	0	+	+	+	+	+w
IV-4	O	+	+	0	+		0	+	+	0	+	+	+	+	+w
R.R., family:															
♂ III-7	A	0	0	0	+		0	+	+	+	+	+	0	+	0
♀ III-8	B	+	+	0	+		not tested								
IV-12	A	+	+	0	+		+	+	+	0	+	+	+	+	+

The genotypes in the mating I-1 x I-2 are $Ve^{a}Ve \times VeVe$ because at least three of their children are Ve (a—) and of genotype *VeVe*. The estimated incidence of this mating is $0.0392 \times 0.0004 \times 2 = 0.00003136$ or 0.003136 per cent. Consanguinity is denied in the mating I-1 x I-2 as well as in II-1 x II-2, the parents of the propositus, both of whom are dead. Each parent in the latter mating must contribute one *Ve*

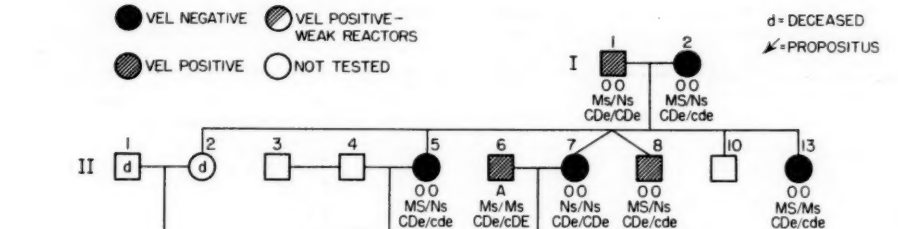

FIG. 1. This figure shows the ABO, Rh, MNSs and Ve[a] types of this family.

gene; II-2 may have been of genotype *VeVe* because three of her children and three of her four sibs tested are Ve (a—) and of genotype *VeVe*. In short, the *Ve* gene is introduced from random sources in at least two different matings in this family tree. Furthermore *Ve* must have been present in one of the parents of I-1 and in both parents of I-2.

Information concerning association with the MNS system is provided in generations I and II. I-1 is heterozygous for Ve[a] and

TABLE 4

Patient	Author or investigator	Blood group and Rh type	Comments
1. M.V., female	Sussman and Miller[4]	O Cde/cde	
2. T.M., female	Levine, P., *et al.*[2]	A CDe/cde	Hemolytic properties.
3. M.B., female	Levine, P., *et al.**	A CDe/CDe	Hemolytic properties; no compatible sibs.
4. W.D., male	Bradish and Shields[1]	A C[w]De/cde	Hemolytic properties; no compatible sibs.
5. G.F., female	Levine, P., *et al.***	O CDe/cde	No relatives.
6. Woudenberg	van Loghem and van der Hart[5]	O —	—
7. W., female	Strickel, Brown and Jack[3]	O CDe/cDE	Compatible sibs. Linkage excluded with ABO, Lewis and Kidd.
8. Stu, female	Allen, F. H., Jr.***	O CDe/cde	Active in saline at 37 C.
9. L.J., female	This report	A cde/cde	Hemolytic properties. Subgroups Ve[a] antigen independent of MNS system.

* Submitted by St. John's Hospital, Santa Monica, California.
** Submitted by American Red Cross, Los Angeles, California.
*** Personal communication.

MN systems. The paternal Ve travels with Ns in II-5 and II-7 and with Ms in II-13; in II-8 the paternal Ve^a travels with Ns. These data quite surely indicate that Vel is not in the MN system. Since I-1 is heterozygous for P and Ve^a, there is a linkage count in this family, the value being 2:0. No further information regarding the inheritance of Ve^a is provided by the blood types of the family. It should be pointed out that all six of the Ve (a—) bloods tested with anti-Fy^a were Fy (a+). In the case of Strickel, Brown and Jack,[3] the propositus had a compatible sister and these workers reported that Ve^a did not belong to the ABO, Lewis or Kidd blood group systems.

In the reactions with several specimens of anti-Ve^a, it was observed that some bloods reacted stronger than others both in hemolysis and agglutination. More specifically, strong reactions were observed in two cases, IV-1 and IV-12, both offspring having one Ve (a—) parent of genotype *VeVe*. Five of the offspring in generation IV must be heterozygous (genotype Ve^aVe) so that dosage is excluded as the cause of the stronger reactions in IV-1 and IV-12. The effect was not always clear cut. We are accepting the explanation of van Loghem and van der Hart[5] that the differences in the intensity of Ve (a+) reactions may be explained on the basis of subgroups, the stronger termed Ve (a_1) and the weaker Ve (a_2). These observations are based on his absorption and elution experiments. The more strongly reacting blood, Ve (a_1) was shown to absorb more completely and the weaker blood, Ve (a_2), absorbed less leaving behind activity for the stronger reacting blood, Ve (a_1), in a manner somewhat analogous to the A_1, A_2 relationship.

A list of published and unpublished cases of anti-Ve^a is presented in Table 4.

From a practical point of view this study illustrates the importance of looking for compatible donors among members of the family group. In the seven Ve (a—) individuals listed in Table 2 only the propositus has anti-Ve^a. Accordingly, anti-Ve^a is an "immune" antibody which has hemolytic properties such as anti-Tj^a (anti-P) and anti-Le^a. To our knowledge hemolytic disease of the newborn due to anti-Ve^a and anti-Tj^a has not been reported, and it is not yet established that anti-Le^a is responsible for hemolytic disease.

Summary

A family with seven Ve (a—) individuals in three generations is reported. Anti-Ve^a was found in the serum of the propositus only. The study indicates that Ve^a is not in the MNS system. The serum possessed hemolytic properties. The findings in this family tend to confirm van Loghem's hypothesis that there are subtypes of the Ve^a antigen.

Acknowledgment

We thank Drs. J. C. Henderson and William P. Creger of Stanford University School of Medicine, San Francisco, California who submitted the original specimen in this case and subsequently arranged for bloods of the family of the propositus to be tested by us.

We are deeply grateful to the members of this family, many of whom were called upon to submit repeat specimens, for their splendid cooperation.

We are indebted to the following who contributed their services in obtaining bloods of various members for this study: James W. Elliott, M.D., Placerville, California; Mr. E. G. Parker, M.T., North Bend Medical Group, North Bend, Oregon; Mrs. F. H. Marnette, Sacramento Medical Foundation Blood Bank, Sacramento, California.

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Studies of Blood Group Antibodies*

V. Fractionation of Examples of anti-B, anti-A,B, anti-M, anti-P, anti-Jk^a, anti-Le^a, anti-D, anti-CD, anti-K, anti-Fy^a, anti-s and anti-Good

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Examples of blood group antibodies were subjected to density gradient ultracentrifugation and anion-cation chromatography on modified cellulose adsorbents and three fractions were collected. The first fraction owed its serologic activity to the presence of γ_2 globulins wih a 7S sedimentation constant; the principal serologically-active component in fraction two was γ_1 globulin of the 7S class; in most cases the serologically-active component of the third fraction was γ_1 globulin of the 19S class. Two anti-A donors were found to have most of the serologic activity in fraction three before injection of blood group substance and a marked rise in fractions two and three after stimulation. In anti-M, anti-P and saline anti-D sera the serologic activity associated with S19 globulins equalled or exceeded the activity with the S7 globulins. Group O serums were studied before injection of A and B substances and S7 activity was preponderant in 48 per cent whereas post-immunization it was preponderant in 88 per cent. Fraction one activity was also preponderant in examples of anti-K, anti-Fy^a, anti-s, anti-Jk^a and anti-Good. Those antibodies which are most likely to cause erythroblastosis fetalis are those which are characterized by a predominance of the S7, γ_2 component.

IN PREVIOUS publications[1, 2] we have reported the separation of anti-A, anti-B and anti-Rh blood group antibodies into various molecular types by means of anion-cation exchange chromatography on modified cellulose adsorbents (DEAE- and CM-cellulose). In other publications,[10, 11] we have described results of use of anion exchange chromatography followed by ultracentrifugation in a sucrose gradient in the study of physicochemical differences between isohemagglutinins anti-A,B, as opposed to anti-A or anti-B. Speer, Prager, Kelley and Hill[12] and Fahey[4] also have applied anion exchange chromatography to the problem of characterization of blood group antibodies, and Fudenberg and Kunkel[7] have used the density gradient system to classify various blood group antibodies according to size. The reported results are in general agreement with ours.

Previous studies now have been extended to include examples of rarer blood group antibodies. It is the purpose of the present paper to report results of these investigations.

Materials and Methods

Fresh serums were obtained from a number of sources: mothers of infants with erythroblastosis fetalis, patients referred to the Hospital of the University of Pennsylvania because of transfusion reactions, donors purposely immunized with incompatible red blood cells or with blood group substances, and apparently normal individuals found to have antibodies in course of routine prenatal studies or cross-matching procedures. In two cases, raw serums were purchased from commercial sources and stored in frozen form prior to examination.

Anion exchange chromatography was carried out as previously described[1, 2] with two exceptions. One serum (anti-Jk^a) was found to be too labile to withstand dialysis

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and slow elution procedures. Accordingly it was placed without dialysis on a DEAE-cellulose column equilibrated with 0.1M phosphate buffer *p*H 5.9. The proteins corresponding to Fractions 1 plus 2 were removed with the same buffer; the proteins corresponding to Fraction 3, plus "euglobulin" were removed with 0.4M phosphate buffer, *p*H 5.2. A second serum (anti-CD) also lost some activity on dialysis at *p*H 6.3. It therefore was dialysed with .0175M phosphate buffer, *p*H 8 and placed on a DEAE-cellulose column equilibrated with .0175M phosphate buffer, *p*H 8. Coombs and albumin active agglutinins were eluted at *p*H 8 with the same buffer; proteins containing saline agglutinins were removed with 1.2M phosphate buffer at *p*H 7.5. Fractions then were equilibrated at 2° C. with 0.85 per cent sodium chloride solution prior to serologic assay.

Ultracentrifugation in a sucrose gradient was carried out as described previously.[10] Serologic tests in sodium chloride solution, in albumin and by the indirect antiglobulin technic have been described.[1, 2] Tests with papain-treated red blood cells were done by the tube-centrifugation method. The cells were treated with papain according to the method outlined by Stratton and Renton.[13]

Results

With the two exceptions already noted each serum was divided into three fractions by anion exchange chromatography. The first fraction (No. 1) previously had been shown to owe its serologic activity to the presence of gamma globulins of low electrophoretic mobility (γ_2) and low sedimentation constant (7S). The second fraction (No. 2) consisted of a mixture of proteins of greater electrophoretic mobility, but previous[10] evidence suggested that the principal serologically-active component was γ_1 globulin of the 7S class. The third fraction (No. 3) also consisted of a mixture of proteins, but the main serologically-active component previously was found to be γ_1 globulin. In most cases this globulin was of sedimentation constant 19S, but serologically-active 7S and non-sedimenting globulins also were present in some serum fractions.*

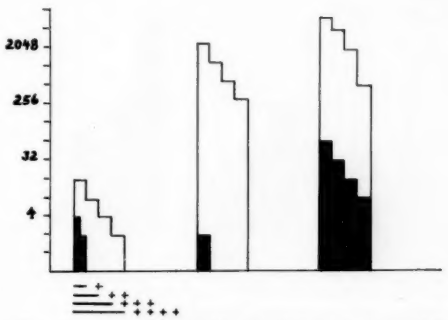

FIG. 1. Chromatographic partition of an anti-B serum before (black area) and after (white area) injection of B substance. Reactivity was maximal in 0.85 per cent sodium chloride solution. The first vertical bar represents Fraction 1; the second, Fraction 2 and the third, Fraction 3. The height of the bar indicates the endpoint of the titer, and the breadth, the degree of agglutination at each point in the sequence of two-fold serial dilutions.

In general, serums were found to fit into two categories—those in which the serologic activity was associated predominantly with gamma globulins of high anionic binding capacity, relatively rapid electrophoretic mobility (γ_1) and high sedimentation constant (19S), and those in which the serologic activity was associated predominantly with gamma globulins of low anionic binding capacity, low electrophoretic mobility (γ_2) and low sedimentation constant (7S). Figure 1 shows a pair of serums typical of the first category. These serums were obtained from a Group A donor before and after receipt of a specific antigenic stimulus in the form of Group B blood group substance. The black areas represent serologic activity before injection; these are superimposed on white areas rep-

* Immunoelectrophoresis shows the chromatographic fractions to contain the following immunoglobulins: Fraction 1—γ; Fraction 2—B_{2A} and γ, Fraction 3—B_{2M}, B_{2A} and γ. It should not be concluded that serologic activity in Fraction 2 or 3 is necessarily always due to the same immunoglobulin.

FIG. 2. Chromatographic partition of an anti-M serum. Reactivity of all three fractions was maximal in 0.85 per cent sodium chloride solution. Scheme of representation same as for Figure 1.

-resenting serologic activity after injection. The first vertical bar represents the titer of Fraction 1, the second, the titer of Fraction 2, and the third, the titer of Fraction 3. The height of the vertical bar represents the serologic titer; the breadth, the degree of agglutination, ranging from one-plus to four-plus, at any point in the sequence of serial two-fold dilutions.

It will be noted that before injection of blood group substance, practically all of the antibody activity was found in Fraction 3. A challenge dose of antigen evoked a brisk response, with a slight rise in the antibody content of Fraction 1, a striking rise in Fraction 2, and a marked increase in Fraction 3. The end result was still a predominance of Fraction 3 over Fractions 1 and 2 in antibody content, as measured by

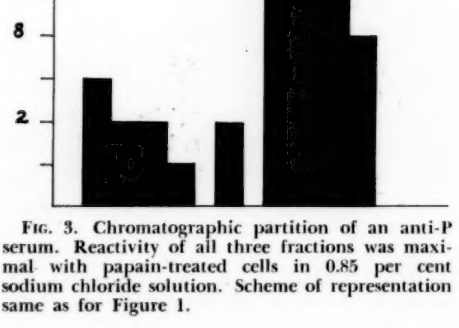

FIG. 3. Chromatographic partition of an anti-P serum. Reactivity of all three fractions was maximal with papain-treated cells in 0.85 per cent sodium chloride solution. Scheme of representation same as for Figure 1.

titer. Ultracentrifugal analysis showed the serologic activity of Fraction 3 to be associated entirely with gamma globulin of sedimentation constant 19S. Study of 25 non-group-O subjects before injection of blood group substance and 31 subjects after injection of blood group substance showed that S-19 activity was preponderant in 80 per cent of the pre-immunization series and preponderant in 90 per cent of the post-immunization series.[11]

Figure 2 shows a similar pattern with regard to an anti-M serum encountered in course of routine crossmatch procedures. Reactions were maximal in sodium chloride solution, and addition of antiglobulin serum depressed the titer in all three fractions.

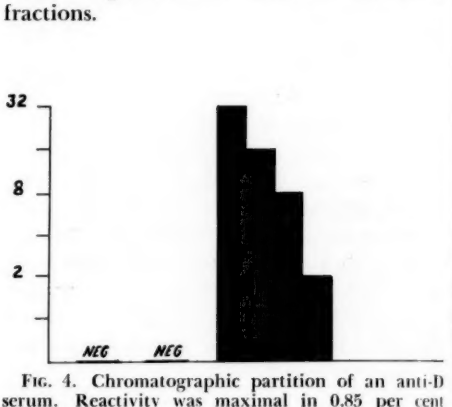

FIG. 4. Chromatographic partition of an anti-D serum. Reactivity was maximal in 0.85 per cent sodium chloride solution. Scheme of representation same as for Figure 1.

Figure 3 shows the distribution of molecular types in an anti-P serum also encountered in routine blood banking procedures. Reactions were maximal in the cold (8° C.) with papain-treated red blood cells.

Figure 4 shows results of fractionation of an anti-D serum which reacted maximally in sodium chloride solution. The patient had been followed for 14 years in various prenatal clinics in Philadelphia. Her anti-D titer apparently had fluctuated between 1:32 and 1:128. She had had five D-positive infants in that period, none of

whom had clinically apparent hemolytic disease of the newborn and all of whom appeared to develop normally. Various other saline-active anti-Rh serums we have studied have shown a similar pattern, with the proportion of Fraction 1 varying, but never exceeding the titer of Fraction 3. When activity was found in Fraction 1, it was detectable only by Coombs test. Results of fractionation of other Rh serums have been reported previously.[2] Severity of the hemolytic disease in the newborn in cases of D sensitization has been more closely correlated with the titer of Fraction 1 than with the titer of Fraction 3.

The preceding figures have been representative of serums in which serologic activity associated with S19 globulins equalled or exceeded the serologic activity associated with S7 globulins. Figure 5 shows typical serums belonging to a second category, that is, serums wherein the serologic activity of Fraction 1, representing globulins of γ_2 electrophoretic mobility and sedimentation constant 7S, predominate over the heavier, more rapidly migrating molecular types. The serums shown in Figure 5 were obtained from a Group O donor who had received B substance.* The vertical bars represent maximal titers. In the case of Fraction 1, the maximal titer was obtained by the antiglobulin test; the titers of Fractions 2 and 3 were maximal in sodium chloride solution. It is apparent that in this serum, serologic activity is spread almost equally among the molecular types and that after antigenic stimulus the response is more pronounced in Fractions 1 and 2 than in Fraction 3.

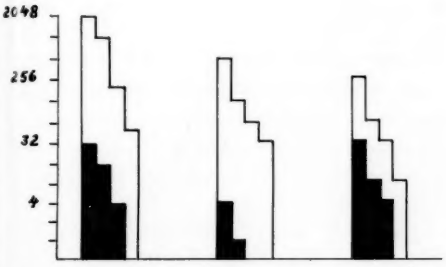

FIG. 5. Chromatographic partition of an anti-A,B serum before and after injection of blood group substance. Reactivity of Fraction 1 was maximal by antiglobulin test; of Fractions 2 and 3, in 0.85 per cent sodium chloride solution. Scheme of representation same as for Figure 1.

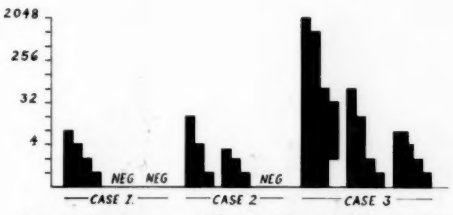

FIG. 6. Chromatographic partition of three anti-K serums. Reactivity was detectable in all three fractions only by albumin or antiglobulin tests. Scheme of representation same as for Figure 1.

Ultracentrifugal analysis confirmed the great heterogeneity of molecular types in this serum. Serologically active proteins of the 7S class were found in all three fractions and Fraction 3 also contained serologically active non-sedimenting proteins as well as serologically active proteins of the 19S class.

Fraction 1 of this serum also contained "cross-reactive" antibody; that is, Group B cells exposed to Fraction 1 gave up, on

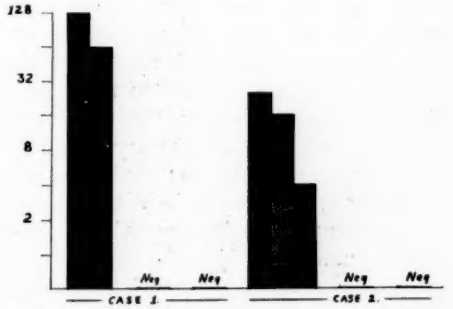

FIG. 7. Chromatographic partition of two anti-Fy^a serums. Reactivity was detectable only by antiglobulin test. Scheme of representation same as for Figure 1.

* Knickerbocker Biosales, Inc., New York City. The B substance also contained a small amount of A substance.

FIG. 8. Chromatographic partition of an anti-s serum. Reactivity was maximal by antiglobulin test. Scheme of representation same as for Figure 1.

elution, agglutinins which reacted with Group A cells as well as with Group B cells.

In all, 25 Group O serums were studied before injection of A and B blood group specific substances, and 34 cases were studied after injection of these materials. In the pre-immunization series S7 activity was preponderant in 48 per cent of the cases; post-immunization, it was preponderant in 88 per cent of the cases.[11]

Other examples of serums wherein Fraction 1 was predominant are illustrated in Figures 6 through 10. Figure 6 shows three anti-Kell serums. All reacted maximally by Coombs test. The one on the left was from a patient whose husband was Kell-positive, but there was no history of infants with hemolytic disease. However, some years after her last pregnancy she suffered a severe transfusion reaction upon administration of apparently compatible Kell-positive blood. Her serum was studied some weeks post-transfusion. All of the antibody activity was found in Fraction 1. The second anti-K serum was obtained from a woman with rheumatoid arthritis. She had a history of multiple transfusions and the antibody was found in the course of crossmatching procedures. Although her serum contained a high concentration of S19 gamma globulin associated presumably with the disease process, all of her anti-K antibody was associated with Fractions 1 and 2. The third anti-K serum was donated by a woman who had a normal K-positive child followed by an infant with hydrops fetalis. Her last pregnancy had occurred more than 15 years before the specimen under investigation was drawn. Yet, as the illustration shows, her anti-K titer was still very high, and the serologic activity covered a broad spectrum of molecular types. Again, however, Fraction 1 was predominant.

Figure 7 shows two examples of anti-Fy[a] sensitization; both serums reacted only by Coombs test. The first serum was obtained from a man who had had multiple transfusions some months previously. His antibody was found in course of crossmatching procedures prior to a second series of transfusions. The second serum was obtained from a woman who had several grown children, none of whom was available for blood typing. She suffered a severe transfusion reaction after administration of apparently compatible blood. The specimen illustrated was drawn while she was undergoing treatment for the transfusion reaction. The antibody in both cases was confined to Fraction 1.

Figure 8 shows an anti-s serum. This serum was described by Levine, Kuhmichel, Wigod and Koch[9] in 1951. The donor had had one infant affected with erythroblastosis fetalis, who recovered with mild neurologic sequelae. This woman's antibody also was confined to Fraction 1.

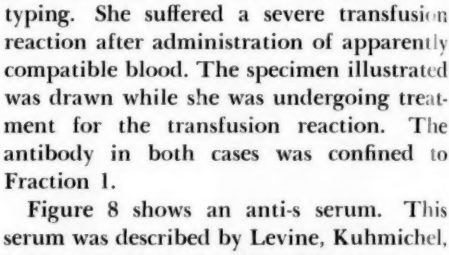

FIG. 9. Chromatographic partition of anti-Good serum. Reactivity was maximal by antiglobulin test but Fraction 1 displayed strong saline activity. Scheme of representation same as for Figure 1.

Figure 9 shows a serum only recently described by Frumin, Porter and Eichman.[6] This serum, named anti-Good, was from a woman who had had four babies with severe hemolytic disease of the newborn. The serum is interesting because it was almost as reactive in saline as by antiglobulin test, and strong saline activity appeared in Fraction 1. This is the only frankly immune serum we have encountered (apart from the ABO series) where saline activity has appeared in this fraction.

The one example of anti-Jk^a available to us was fractionated according to the technic described under Materials and Methods. The activity was recovered in Fraction 1. Reactions were good with the anti-globulin test, but slightly stronger with papain-treated red blood cells allowed to react with the fraction and subsequently exposed to anti-globulin serum. Chromatographic partition is shown in Figure 10.

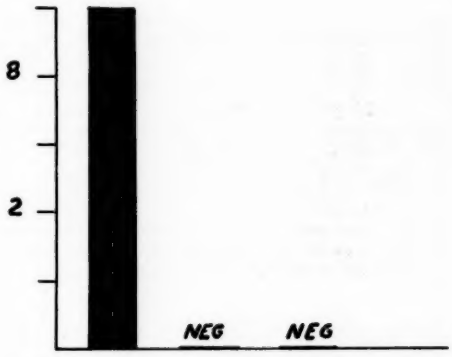

FIG. 10. Chromatographic partition of anti-Jk^a serum. Reactivity maximal by papain-antiglobulin test. Scheme of representation same as for Figure 1.

Attempts to fractionate anti-Le^a serums met with even greater difficulties than those attending fractionation of anti-Jk^a. Results were not clear cut; in the main, the serologic activity appeared to be associated with 19S proteins; however, in one serum examined at least part of the antibody protein appeared to be of the 7S class.

Table 1 shows the fractionation of an anti-CD serum by the technic described under Materials and Methods. This serum was from a male volunteer with a transfusion history who had subsequently and over a period of some years periodically received small doses of CD-positive red blood cells to maintain his antibody titer. The reactivity of his serum with Rh_2 cells had markedly and progressively changed. Originally the serum was saline-active; later a prozone appeared in saline and finally the saline reactivity disappeared. After a longer period of time a prozone appeared in albumin. Fractionation again disclosed the saline agglutinin and demonstrated the great heterogeneity of molecular types composing the antibody spectrum.

Discussion

Perhaps the most striking correlation suggested by this study is the parallelism between those antibodies which are most likely to produce erythroblastosis fetalis, and those antibodies which are characterized by a predominance of the S7, γ_2 component. The latter group includes O anti-A, O anti-B, anti-D, anti-Kell, anti-Jk^a, anti-s, anti-Fy^a, and anti-Good. Antibodies which are composed predominantly of the S19, γ_1 component such as A anti-B, B anti-A, and some examples of saline anti-D are unlikely to be associated with severe erythroblastosis fetalis. This would tend to support the thought that S7 protein, which can pass through the placental barrier with ease, is of greater significance with respect to erythroblastosis fetalis than is S19 protein which cannot pass easily from maternal to fetal circulation.[5] In this connection it is also interesting to note the recent report of Lee, Takahashi and Davidsohn who observed that the anemia-producing factor of rabbit anti-mouse erythrocyte serum was associated mainly with γ_2 globulin fractions, rather than with γ_1 globulin fractions, even though the latter contained *in vitro* the major portion of the hemolysin and saline agglutinins.[8]

TABLE 1. *Reactivity of Anti-CD Serum Before and After Fractionation*

With	Titers before fractionation		Titers after fractionation			
			Front-running component		Late-running component	
	in saline	by Coombs test	in saline	by Coombs test	in saline	by Coombs test
rh′ cells	1:32	1:512	0	1:128	1:64	1:128
Rh_1 cells	0	1:4096	0	1:512	1:256	1:512
*Rh_2 cells	0	1:4096	0	1:512	1:256	1:512

* Serum before fractionation had a strong prozone in albumin to a dilution of 1:8. Prozone after fractionation was weaker and extended to a dilution of 1:4.

The previously observed association in the ABO system and Rh systems of Coombs enhanced activity with the S7, γ_2 antibody component, and maximal avidity in saline with the S19, γ_1 component is extended by this study. In the case of each of the antibodies studied the same relation between physicochemical character and serologic activity was observed. In the ABO system the S7, γ_2 component, although enhanced in its agglutinin titer by Coombs serum, likewise exhibited activity in saline. The anti-Good serum showed a similar phenomenon; in all other frankly immune serums this component showed activity only in the presence of antiglobulin serum.

The results of this study suggest that increasing heterogeneity of antibody molecular types is seen with increasing response to antigenic stimulus. In the case of the three anti-Kell serums, the patient with the poorest reproductive history and highest anti-K titer had the broadest spectrum of antibody types insofar as anionic binding capacity was concerned.

Heterogeneity of a different type is seen in the ABO system where early antiserums contained proportionately more of the S19, γ_1 component than later antiserums which tend to develop more of the S7, γ_1, and S7, γ_2 components. The same phenomenon is displayed by the anti-CD serum shown in Table 1.

The results set forth in Table 1 throw some light on the question of what happens when saline reactivity is replaced by Coombs-active or albumin-active antibody. It is apparent that in this serum the saline-active component was present, but masked. Whether the masking was due to inhibiting factors such as loose protein-protein binding or to competition for antigenic sites cannot be determined from the data presented, but the latter seems a more likely possibility.

Examination of the fractions with G-specific cells might yield further information as to the nature of anti-G; there is nothing in the present data which is in disagreement with the concept of Allen and Tippett regarding the nature of the G antigen-antibody relationships.[3]

Summary

The technics of anion exchange chromatography and density gradient ultracentrifugation have been applied to the study of a number of the rarer blood group antiserums, and the results are compared with observations on the ABO and Rh systems. Serums from individuals with normal immunologic and clinical histories displayed some heterogeneity of antibody molecular types, but in general the heavier molecular weight (19S) class was predominant. Serums from subjects with a history of exposure to antigenic stimuli (incompatible pregnancy, incompatible blood transfusion or the injection of incompatible blood group substance) tended to display increased heterogeneity in response to some antigens. This was particularly marked in cases of Rh, Kell and

ABO immunization. In all "immune" sera, however, serologic activity associated with proteins of the lower molecular weight class predominated over serologic activity associated with proteins of the 19S class.

The predominance of lower molecular weight antibodies in the maternal serum can be correlated with occurrence of erythroblastosis fetalis in the offspring.

Acknowledgments

The authors are indebted to Dr. Fred H. Allen, Jr., Mr. Russell Fowler, Dr. Lyndall Molthan and Dr. Mary McKee Porter for making serum specimens available to them.

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Short Communication

Note on the Inheritance of Wr^a*

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LINKAGE counts (1-4) have shown that the antigen Wr^a apparently is not part of the ABO, MN, P, Rh, Lewis, Duffy, or Kidd systems and is not produced by a gene on the sex chromosome. A family was encountered (Fig. 1) that confirms independent segregation from the genes of the MN, Rh, and Duffy systems (linkage counts of 6:2, 1:1, and 1:1, respectively), while seeming to show also that Wr^a does not belong to the Lutheran blood-group system.

The relevant portion of the pedigree is shown in the figure. III-2 cannot have a single gene that produces both Wr^a and Lu^a since she inherited these from different parents. If Wr^a and Lu^a were produced by allelic genes, III-2, having both, could not have given both to her son IV-1, or neither to her son IV-2, barring the possibility of chromosomal abnormalities (such as (a) trisomia in III-2; (b) trisomia in IV-1 and monosomia in IV-2; (c) abnormal crossover, with addition, in IV-1, and presumably a second abnormal crossover with deletion in IV-2; or (d) a somatic crossover, abnormal at that, in III-2). All persons are normal, a strong argument against chromosomal abnormalities. Thus, there is every reason to believe that Wr^a and Lu^a are not produced by allelic genes. On similar grounds, close linkage between *Lu*^{a} and *Wr*^{a} would seem most unlikely since this would require that both IV-1 and IV-2 be the result of crossovers, or that some even less likely genetic event had occurred.

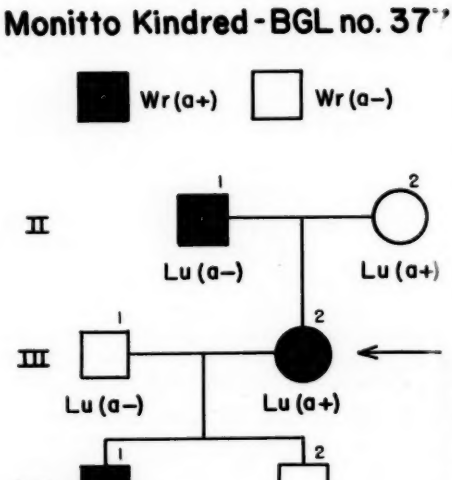

FIG. 1.

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Second Example of Hemolytic Disease of the Newborn Caused by Anti-Jk^{b}*

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Mild hemolytic disease developed in the second child of a woman who gave no history of transfusions. The antibody was identified as anti-Jk^{b}. The antiglobulin method gave the strongest reactions but the antibody also agglutinated test cells in saline at room temperature and at 37 C. No difficulty was encountered in determining the presence of the Jk^{b} antigen in cord blood erythrocytes.

THE KIDD blood group system was discovered by Allen, Diamond and Niedziela in 1951. The antibody, anti-Jk^{a}, found in Mrs. Kidd's serum recognized the new antigen Jk^{a} and distinguished two phenotypes, Jk (a+) and Jk (a—).[1] The expected antithetical antibody, anti-Jk^{b}, was found by Plaut, Ikin, Mourant, Sanger and Race in 1953.[4] Most of the examples of anti-Jk^{b} reported have occurred following repeated blood transfusions and were accompanied by other antibodies. Rosenfield, *et al.*,[6] reported a transfusion reaction where the "only intragroup antibody formed following a series of transfusions" was anti-Jk^{b}. Levine, *et al.*,[3] reported the only other "pure" example of anti-Jk^{b}. The clinical importance of this antibody is not fully established as yet, although some mild hemolytic transfusion reactions have been attributed to it.[6, 7]

The first case of hemolytic disease caused by anti-Jk^{b} was reported by Kornstad and Halvorsen in 1958.[2] In their case, the anti-Jk^{b} was accompanied by a probably naturally occurring anti-S which did not play an etiologic role because the infant was S-negative.

We are presenting the second example of hemolytic disease of the newborn caused by anti-Jk^{b} which, in this instance, was not accompanied by any other blood group antibody.

Case Report

Mrs. T., a 25-year-old, gravida II, white housewife was admitted to the maternity ward of our hospital for the delivery of a full term baby. Her first pregnancy was uneventful and terminated with the delivery of a healthy infant. She had no previous miscarriages or abortions. There was no history of transfusions of blood, plasma or any blood derivatives.

She delivered a normal seven pound, 13 ounce male infant. Routine postnatal serology was done within a few hours after delivery and an atypical antibody was found which reacted with all the red cells containing the Jk^{b} antigen. The cord blood contained the same antibody. The direct antiglobulin test was positive and

* Received September 1, 1960; accepted November 16, 1960.

TABLE 1. *Blood Groups of Family T.*

Mrs. T.	A_2B	MN	S+	s+	P+	Rh_1Rh_2	Le (a+)	Fy (a+)	K−	k+	Jk (b−)
Mr. T.	O	MN	S−	s+	P−	Rh_1rh	Le (a−)	Fy (a+)	K−	k+	Jk (b+)
1st Child	B	M	S−	s+	P−	Rh_1rh	Le (a−)	Fy (a+)	K−	k+	Jk (b+)
2nd Child	B	MN	S+	s+	P−	Rh_2rh	Le (a+)	Fy (a+)	K−	k+	Jk (b+)

the total bilirubin 1.4 mg. per cent. The mother was group AB, Rh-positive and the infant group B, Rh-positive. The infant became slightly jaundiced within the first 24 hours and the icterus became more intense later. Physical examination revealed nothing abnormal; liver and spleen were not enlarged. The infant was discharged with the mother five days after birth.

Additional Data

The serum of Mrs. T. was set up against a panel of O cells* at 4, 22, and 37 C in saline and incubated for one hour. Definite agglutination occurred at 37 C and at room temperature (22 C.) with cells which contained the Jk^b antigen. There was no reaction against the same cells at 4 C. The agglutination was much stronger when the indirect antiglobulin test was carried out on these cells. The patient's serum did not react against her own cells either in saline or by the indirect antiglobulin test.

Titration gave the following results: in saline 1:1 and by the indirect antiglobulin test 1:4. Three days after delivery the titers were 1:1 in saline and 1:16 by the indirect antiglobulin test. The titers were the same with serum obtained three months after delivery. The test cells employed in all these titrations were of the phenotype Jk (a−b+) but there was no difference in titer when the tests were repeated employing test cells of the phenotype Jk (a+b+). Similarly there was no difference in titer when these test cells were trypsinized. Controls consisted of the patient's own cells and another random Jk^aJk^a cell, and a known anti-Jk^b reagent with the patient's cells and a Jk^bJk^b cell.

Other blood group antibodies were excluded by the negative reactions against the panel cells which did not contain the Jk^b antigen and a few random Jk^aJk^a bloods. It will be seen from the blood groups of the family T. that it is unlikely that Mrs. T. would develop other isoantibodies as a result of pregnancy (Table 1).

The identification of the antibody and the fact that the anti-Jk^b in the patient's serum is in a "pure" form was confirmed by Dr. Philip Levine's laboratory.

Discussion

The diagnosis of hemolytic disease was based on the positive direct antiglobulin test on the cord blood, the appearance of jaundice within the first 24 hours of life, the elevated bilirubin levels and the relatively low hemoglobin values observed (Table 2).

Race and Sanger[5] mention the absence of any reference regarding tests on cord or fetal blood with anti-Jk^b, and we were also unable to find references on this subject in the cited works. It might be interesting to note that we did not experience any difficulty in determining the presence of the Jk^b antigen in the cord blood cells in this case and in ten other cord bloods. Blood obtained from the same infants a few days later confirmed the previous findings.

* Knickerbocker Pano-cells.

TABLE 2. *Hematologic Data of Infant*

Hours after birth	Hemoglobin Gm. %	Bilirubin mg. %		Reticulocyte count
		Direct	Indirect	
Cord	Not tested	0.0	1.4	
25	15.2	0.5	5.7	4.6%
42	14.4	1.0	8.0	
57	14.8	1.0	7.5	3.4%

Summary

A case of hemolytic disease of the newborn due to anti-Jk^b is presented. The antibody was found in "pure" state in the mother's serum. No difficulty was encountered in determining the Jk^b group of a limited number of specimens of cord blood.

Acknowledgment

The cooperation of the Ortho Research Foundation's Laboratory in confirming the identification of the antibody is gratefully acknowledged.

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Special Article

Achievements and Unsolved Problems of Blood Group Anthropology*

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WHEN in 1900 Landsteiner[10] discovered the ABO blood groups no one could have foreseen the wide repercussions of his discovery upon the whole of biological science. Even its simpler and more direct implications were slow to be appreciated. For instance in 1904, Nuttall,[18] another pioneer of immunology, though referring in the same book to Landsteiner's first classical blood group paper in connection with the discovery of "anti-ferments," apparently did not realize that the blood groups had any possible connection with transfusion, and could write:

"There is no evidence that the sera of any human races are haemolytic for the blood corpuscles of other races of man, in proof of which we have a large experience with regard to transfusion. Transfusion from the blood of one race into that of another has not been followed by any ill effects."

In parentheses, however, I feel I ought to add: "Have we ourselves never been similarly blind in failing to recognize an important new discovery?"

As is well known, the first deliberate investigation into the frequencies of the blood groups in different races was carried out by Professor and Mrs. Hirszfeld,[8] and the results were published in 1918 and 1919. These at once showed that wide and statistically highly significant frequency differences did exist, and during the next two decades numerous papers were published giving the blood-group frequencies of different populations, which were summed up by Boyd[3] in his classical monograph in 1939. Meanwhile Landsteiner in collaboration with Levine[11] had discovered the MN and P blood group systems; also, in collaboration with Strutton and Chase,[12] he had found the Hunter antigen and noticed its special association with Negroes. They also noticed its connection with the MN groups, which is of the kind now widely regarded as due to close linkage of the genes concerned. It may, therefore, be claimed that multiple allelomorphism of the type now mainly associated with the Rh groups was in fact discovered in 1934 by Landsteiner and his colleagues though they did not at the time fully realize its implications.

Later, in 1940, Landsteiner and Wiener[13] announced their finding of the Rhesus or Rh blood groups, a discovery with consequences in medicine and genetics comparable in importance to those following the original discovery of the ABO groups.

Next the remarkable complexity of the Rh groups was uncovered, initially mainly by Wiener, Race and Levine. The continuing discovery, by numerous workers, of new antigens and new complexities in the serology and genetics of this system have not ceased to tax the ingenuity of all concerned with their genetical interpretation.

Mainly as a result of the discovery of the Rh groups, clinicians and pathologists have become increasingly aware of the practical importance of iso-immunization, and this has led to the discovery of new, genetically independent, blood group systems in almost bewildering profusion, so that eleven such systems have now been recognized for which some human populations are polymorphic, quite apart from familial systems where members of only a single family or a few families throughout the world are known to possess a particular gene and antigen, or to lack an antigen otherwise universally present. It may be that, as happened in the case of the Diego system, other genes thought at the time of discovery to be rare and familial will later prove to be relatively common in some populations.

As recently as 15 years ago, few well-defined polymorphisms other than those of the blood groups were known in man. Now the number of known polymorphisms, mainly biochemical, is large and growing, and many of the systems concerned show important gene frequency differences between different races and populations. The most prolific fields of discovery in this respect have been those of the hemoglobins and the plasma proteins.

The achievements of blood-group and other genetical investigations applied to anthropology

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have been so numerous and varied that I can, in the time at my disposal, mention only a few examples. I wish, rather, to emphasize the gaps in our knowledge and the lines of enquiry which appear to me most likely to be fruitful.

I have discussed elsewhere the question of the mechanisms whereby human populations have come to differ in their gene frequencies for the different blood group systems: there can be little doubt that, as Sir Ronald Fisher and Dr. E. B. Ford especially have long held, the main influence is that of natural selection, though in some cases genetic drift is probably important. However, selection may take place at different rates for different systems (or indeed for the same system in different places). Those systems which are subject only to slow selective changes and which give rise to very stable polymorphisms will be of most use in tracing ancient population movements, and it may be difficult to find out what are the selective processes affecting them, while more rapidly selected systems will be of use only in tracing comparatively recent events, but may be of great interest in the direct investigation of natural selection. Similar considerations also apply to other polymorphisms such as those affecting the hemoglobins and the plasma proteins.

There are indications that the ABO groups are subject to more rapid selection than at any rate those of the Rh and MN systems, but even ABO frequencies seem to be stable over periods of up to one or two thousand years, in contrast to the hemoglobins which are probably subject to much more rapid selective processes.

For the hemoglobins, however, or at least for the sickle-cell and normal adult types, we can be reasonably certain that in certain environments a state of balanced polymorphism exists, with the heterozygote having a selective advantage over both homozygous types. For the blood groups, however, there is no evidence that any heterozygote has an advantage over the corresponding homozygote and, in the particular case of the ABO groups, there is little if any difference in behavior *in vitro* between AA and AO or between BB and BO, and it is difficult to imagine, therefore, how the heterozygotes could enjoy any marked advantage *in vivo*. Nevertheless, in the absence of evidence to the contrary, one must assume provisionally that the observed polymorphisms are in or near a stable state of balance, and look for the mechanisms which are needed to support such a state.

The main application of blood group investigations to anthropology consists in attempting to trace ancient population movements, and mixing of populations. Though gene frequencies are thought to be more stable in some blood group systems than in others, in none do they appear too unstable to be used for these purposes.

In the time at my disposal I can mention only a few of the more interesting examples of such applications. In the discussions which follow, I have drawn considerably on my own compilations of data[16, 17] and have not, in general, given separate references to the very numerous original sources. To begin with Europe, one interesting discovery is the similarity in blood group frequencies between the Scots, the Irish, the North Welsh and the Icelanders, all of which populations have a much higher proportion of group O genes and a lower one of A than the present population of most of Scandinavia, from whence the Vikings are known to have sailed to Iceland. It has been suggested that the ancestors of the Icelanders came mostly from the British Isles and that the Vikings, who wrote the Sagas which tell the history of Iceland, were only a small ruling caste. However, it appeared more likely that the majority of the original Icelanders did come from Scandinavia, the present inhabitants of the latter country being largely descended from more recent immigrants. This view is supported by the work of Beckman[1] who has found in Sweden important enclaves of people with a high frequency of O and with other characteristics suggesting a close relationship with the ancient peoples of the western fringes of Europe. Here we are dealing with events of the last few thousand years and can safely use all the blood group systems.

On the other hand, in Africa, with a wide variety of ABO frequencies, we find a fairly constant and characteristic picture for each of the systems Rh and MNSs. This picture is shown not only by the tribes generally regarded, in the broad sense, as Negro, but also by the physically very different Northern Nilotes, and by the unique Bushmen and Hottentots, formerly regarded by many as totally distinct from the Negro race. We might, indeed, regard the observed facts as a result of convergent evolution operating on peoples of completely different origins, but Africa south of the Sahara is sufficiently diverse to make this an improbable explanation, and it is much more likely that the Rh and MNSs frequencies represent the original composition of a generalized African

population, upon which more recent selection in the various environments has superimposed the existing variety of ABO frequencies. If there were time I could give you many other examples of positive conclusions about peoples, deduced from a knowledge of their blood groups. But I want now to turn to some unsolved problems, and especially to a few where we can hope that further investigation will help to solve them.

Meeting as we are now doing on the shores of the Pacific Ocean, we find our thoughts drawn to the problem of the peopling of the Americas, and to trans-Pacific human migrations in general. Archaeology has told us much, and can no doubt tell us much more, about prehistoric man on this continent. Physical anthropology tells us that American Indians and Eskimos are Mongoloids, and there can be no reasonable doubt that their ancestors came from Asia. We can even make reasonable conjectures as to what present Asiatic peoples are the nearest relatives of the pre-Columbian peoples of America, but no very precise conclusions have yet been reached. This is the type of problem in which blood groups have often proved helpful in the past, but it must be admitted that they are at the moment far from having solved the present problem.

There is a recognizable pattern about each side of the Pacific when they are considered separately. On the American side, with a wide diversity of ABO frequencies, the North and South American Indians and the Eskimos share exceptionally high frequencies of the M and R_2 (cDE) genes. North American Indians have a variable A and little or no B, South American Indians have little or none of either A or B; Eskimos have high A and low B.

The Chinese, Japanese and Koreans have a high A and B, with only moderate frequencies of R_2 and of M. The Japanese have a frequency of R_2 which is somewhat elevated but barely reaches the lowest American levels. Tests for MN appear not to have been done on Koreans. The Ainu, with an exceptionally low M frequency, throw no light on the subject.

Thus the three "classical" blood group systems, ABO, MN, and Rh, appear to divide rather than unite the peoples on the two sides of the ocean. The main uniting factor is the Diego system,[14] for the Di^a antigen is present in North and South American Indians, Chinese and Japanese. Most surprisingly, however, it is almost absent from the Eskimos.

Blood groups having given little in the way of a clue we turn for a moment to geography. The earliest men in America cannot have had ocean-going boats, if indeed they had boats at all. They must have crossed Bering Strait, either by sea or over the ice. Thus one might expect the nearest relatives of the ancient American peoples to be found today in Eastern Siberia. Eskimos are indeed found there, but there are reasons for thinking that their ancestors came fairly recently from America, even though earlier ancestors must have come from Asia. Our present knowledge of the blood groups of the Tungus and Chukchi peoples is slight, but the Soviet authorities are now actively investigating the blood groups of the East Siberians and we may hope for very useful information in a few years' time. At the moment all that can be said is that the Tungus and Chukchis have ABO frequencies which are, on the whole, intermediate between those of the Eskimos and those of the other peoples of eastern Asia, that the Chukchis have only moderate M frequencies, but that the Tungus peoples, if we can generalize from the very small sample of Lamuts tested, have a high frequency of M. The latter fact, together with the rather high frequencies of M found in eastern Europe and southern Asia, suggests that, behind the screen of moderate M frequencies found in the better known parts of eastern Asia there may be a large reservoir of very high M levels related to the similarly high levels found in America.

Here, however, we are entering the realm of speculation, so perhaps we may at this stage proceed to consider another related topic characterized by few facts and many speculations, that of the origin of the Polynesians. It is well known that Heyerdahl[7] thinks that their ancestors came from America, partly from South America and partly from the West Coast of what is now Canada. I think that most archaeologists and anthropologists disagree with this theory, but enough evidence has been brought forward in its favor to make us consider it seriously.

Rather surprisingly, the blood group evidence gives considerable support to Heyerdahl's views. The Polynesians, like both American Indians and Eskimos, have a very high frequency of R_2 and, like the North West American Indians, they have a rather high frequency of A and practically no B. The rather scanty evidence referring to other genetical systems agrees reasonably well with the theory, except for the MN and Diego blood group systems and the secretor factor for ABH blood group substances.

The Polynesians[5, 9, 19, 20, 21] resemble the Chinese and Japanese in having rather more than 50 per cent of M genes. They have nothing like the very high frequencies of M found in the

Americans. However, they have considerably more M than the Melanesians and Micronesians, their immediate neighbors to the West, whose effect may have been to reduce M levels by interbreeding. They also lack the Diego (Di^a) factor present both in the American Indians and in the peoples of Eastern Asia (but very rare in Eskimos). The evidence with regard to secretion is somewhat confusing. There is no doubt that the North American Indians are nearly all secretors. The Maoris show a very high frequency of non-secretors and of the associated Le (a+) blood group. The Easter Islanders[19] have been found to include a very high frequency of the Le (a—) blood group, who are presumably nearly all ABH secretors. However, the specimens, admittedly preserved with the greatest of care, were several months old when tested. I have discussed the problem with Mr. Simmons who undoubtedly carried out the most careful control tests, but I confess that I should prefer to rely on the results of testing fresh specimens.

Apart, therefore, from the secretor factor, and perhaps the Diego blood groups, the genetical evidence on the whole agrees with Heyerdahl's theory. I do not think that it proves the theory right, but it does strongly suggest that there is a considerable and fairly recent element of common ancestry shared by Pre-Columbian Americans and Polynesians.

Since, however, a large part of our information on Polynesian blood groups comes from the Maori, it is important that other Polynesian populations should be thoroughly tested for blood groups and other relevant genetical factors, before they lose their identity by interbreeding, and I would particularly make a plea here for a thorough study of the purest Polynesians available in Hawaii. I should like to see as many tests as possible carried out, but it is particularly important that secretor tests should be done, as well as tests for the Lewis groups.

As I have already indicated, the other region, blood group data from which could help to solve the problem of the pre-Columbian peoples of America, is Eastern Siberia, but the relatively blank area in this part of the map is only part of the much wider region of scanty information covering the whole of Soviet Asia. It will take many years to fill in the blank areas, but Russian anthropologists are now well aware of the need for blood group information and, as I have mentioned, they have now begun an active campaign of research.[15]

There are many other areas in the world where we can hope that more detailed blood group investigations will help to solve anthropological problems; for instance, in Australia there are two schools of thought regarding the fairly considerable variations in blood group frequencies which are found in the Aborigines in different places. Birdsell regards these variations as evidence of an original heterogeneity, whereas I think that Abbie would regard them as resulting from genetic drift. When tests have been carried out for a sufficient number of systems of blood group and other genetical characters, these should show closely related frequency trends if variations are the result of an original heterogeneity, but if the variations are due to genetic drift each system will vary independently of the others.

Instances of relatively straightforward problems of this kind could be multiplied almost indefinitely, but there are a number of more fundamental problems awaiting solution. One of these is that of the evolutionary origin of the blood groups themselves—a problem which transcends anthropology and has indeed implications affecting the whole of biology.

As I have already said, natural selection appears to be the main process which has been responsible for changes in blood group frequency in different populations, and there are indications that some of the systems, especially the ABO, are subject to more rapid selection than others. Associations between particular blood groups and certain common diseases, such as between group O and duodenal ulcer, have given a partial clue to the method of selection, but duodenal ulceration is not a disease with any very obvious immunological background, and I think that the infectious diseases may give us more fundamental information. Stating the problem in its crudest form, we might expect that if a particularly infectious micro-organism carried an antigen related to a particular blood group antigen, then persons who possessed that blood group antigen might have more difficulty than other people in elaborating an antibody to combat the infection. Thus we might find that sufferers from the disease showed a raised frequency of the blood group antigen concerned, as compared with the rest of the population.

Such a mechanism may account for the observations of Clarke, McConnell and Sheppard[4] who have shown that there is a significant deficiency of group O among sufferers from rheumatic carditis, a condition which can arise as a sequel to hemolytic streptococcal infection.

The same idea is at the basis of the work of Helmbold, Pettenkofer and Vogel[6] who have investigated the antigenic composition of the

micro-organisms responsible for cholera, plague and smallpox. They find that the plague bacillus carries an H-like antigen, similar to that found on red cells of all groups but especially those of groups O and A_2. The smallpox virus carries an A-like antigen, while no blood-group-like antigen could be detected in the cholera vibrio. A comparison, upon world maps, of the distribution of past great epidemics with the present distribution of the ABO blood group genes showed that on the whole smallpox epidemics have occurred in regions with a low frequency of A and plague epidemics where the frequency of O is low. It is now proposed to study blood group incidence in actual epidemics. If by investigations of these and other kinds into the relationship between blood groups and various diseases it can be shown to be likely that natural selection due to such diseases has substantially affected blood group frequencies, an important step will have been taken towards understanding why blood groups exist at all. It might be supposed that it would be best for any individual, or any population, to carry few or no red-cell antigens of the blood-group category, but it may be that such antigens are a necessity for life for some reason which we do not at present understand, and that the existing blood-group constitution of a given population is a compromise between this necessity and that of the best possible protection against the prevailing pathogenic micro-organisms.

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Administrative Section

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Planning Your Blood Bank?*

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THE planning of hospital blood banks has been neglected in the past to such an extent that many are inadequate, overcrowded and inefficient. The widespread increase in the number of transfusions and the expanding science of immunohematology have created design problems for the blood bank which were not previously anticipated. Further scientific growth in these important fields may be expected in the future and thus anticipated needs in the physical aspects of this department should be reviewed now.

In order to plan this division many factors must be considered. The function of a blood bank may vary markedly from institution to institution. While some banks are completely integrated and obtain their blood supply by their own efforts, others obtain blood from third parties and serve merely as depots from which to dispense it to the patients.

Size of Blood Bank

The character and quality of the hospital professional services are important. Is surgery abundant or minimal? Does the hospital admit more acute than chronic diseased patients? Is it private, university affiliated or governmental? Does it have large out patient departments (dispensaries, clinics or emergency divisions)? Is there a preponderance of medical, surgical or other types of disease? Is heart or lung surgery practiced?

The geographic location of the hospital plays a role. Rural hospitals may need small blood banks only. Metropolitan hospitals in densely populated, industrial areas often require larger blood bank facilities because of a greater number of traumatic injuries or because they are more active in certain fields—special surgery, neoplastic diseases, etc.

People's personalities are reflected in the blood bank's size. The pathologist's professional and personal attributes, his interest in immunohematology and his aggressiveness in attempting to secure adequate space, equipment and personnel to operate a blood bank are important. The extent of training and experience of the attending staff, the number of certified specialists and the existence of resident and intern training programs are elements which contribute to stimulate the blood bank to improve its services. Their awareness of the need for, as well as of the hazards of, blood transfusions and the degree of enthusiasm or of caution in the use of blood, plays a large role.

The hospital administrator must be acquainted with the importance of this division and furnish adequate facilities in terms of space, personnel and equipment. The size and activity of blood banks will be reflected also by the quality of its medical technologists, technical assistants and other professional and administrative persons.

There are several methods by which one may attempt to obtain information in blood bank design questions. One may make the "grand tour" and see how "George" does it. While

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useful, it may have the disadvantage of propagating errors and examples of past shortsighted or inadequate planning and thus serve to reproduce inefficient, outmoded plans and designs. One may ask for advice, but there are only a few experienced consultants in the field of hospital planning who are adequately versed in the difficult technical and administrative problems of a blood bank. Often a "layout" which seemed adequate at the time it was created becomes too small and unable to cope with increased demands which time and progress of blood banking methods impose upon it.

A more sensible method to determine size would be to calculate from past experience the anticipated number of transfusions, and to project this estimate into the future. To this estimate one must add reserve capacity to be able to accommodate new developments. These will result in changes in indications for transfusions, the manner of blood collection and storage, the duration of storage and the technical procedures required to process blood for transfusion. All plans must contain within them opportunities for change, growth and expansion; we believe the science of immunohematology is in a state of vigorous growth.

The Director of the Blood Bank must review all of the technical and administrative procedures performed in the blood bank and study the most efficient manner in which equipment and facilities are to be arranged in order to eliminate confusion and the waste of space and time in the performance of these procedures. Thus, it is possible to reduce the cost of production by permitting more efficient employment of the staff to perform a larger number of procedures in a given area. The Director must program the requirements of the blood bank in order to advise the architect, who is usually not versed in modern practices of medicine, how to design this division.

The architect's chief responsibility is to solve the problems of an architectural nature which relate the blood bank to the over-all service and utility scheme of the building. He supplies expert opinion on structural and mechanical engineering and budget, interprets building codes and safety and fire underwriting requirements. He suggests methods of construction and service supply, materials, finishes, timing, scheduling and supervision of construction. His duties fall into several stages:

A. Schematic Stage: The architect prepares small scale drawings for the blood bank director and other parties in the hospital to study the relationship between the blood bank and the clinical departments.

B. Preliminary Drawings: Preliminary drawings are made which show major partitions and dimensions and the location of items of laboratory equipment and services to determine the most economical and practical systems of plumbing and heating, electrical wiring, ventilation, air conditioning and other engineering problems. Changes may be suggested at this stage.

C. Working Drawings: Working drawings and specifications show the quality of workmanship and the type of equipment needed. These must be specified and attention to detail is mandatory since changes from these plans and specifications after acceptance of the contract are subject to extra charges.

Location of the Blood Bank

Will the blood bank be responsible for procurement, processing and dispensing of blood or will it have sources other than by its own bleeding activities? If a blood bank is serviced by a third party (American Red Cross, community bank or commercial supplier), the problems concerning the design of a bleeding room will not arise. The problem of controlling donor traffic is simplified and the hazard of hospital acquired infections due to the entry of transient persons is eliminated. If the bank is responsible for procurement of its blood supply, these problems become most important in deciding the location and design of the division.

It is necessary that the blood bank be located in an area where opportunities exist for expansion and redesign without creating significant architectural problems or incurring large expense. It should be in an area of easy egress for transient persons to make donations. It must be accessible to outside parking areas, hospital entrances, elevators and stairways and yet be sufficiently isolated so as to reduce traffic in major hospital corridors and to eliminate overloading of public waiting rooms and reception areas.

The problem of hospital acquired staphylococcus infections requires that public traffic be reduced to a minimum. Thus, the blood bank should not be located in an area which is close to the clinical divisions. First floor or basement locations are satisfactory. The blood bank and the clinical pathology laboratories are closely integrated and should be nearby. The laboratories, however, may be located in a remote portion of the hospital on an upper floor. Thus, the problem of traffic and control of infections

becomes more difficult. In addition one must consider the need of servicing the blood bank at night and on weekends which is often done by laboratory technologists.

Equipment Requiring Services

In programming the blood bank, consideration of the type and amount of fixed and portable equipment requiring one or more services is necessary. Some of these are:

- sterilizers
- water baths
- pipette washers
- telephone system
- heating units
- hot plates
- incubators
- night bells
- ventilators
- laboratory furniture and casework including sinks, counters, cupboards and work benches
- office furniture including desks, files, typewriters, adding machines, copying machines
- electric oven
- distilled water apparatus
- drinking fountain
- hospital page system
- electric wall clocks
- exit signs
- air conditioners
- intercommunication system
- fire alarm system
- refrigerators
- centrifuges
- blood bank refrigerators with temperature recording devices and emergency bells at telephone switchboard to warn of power failure

The proper location for this equipment must be predetermined in order to permit economic installation during construction and to be easily maintained.

The following services are usually required:

- waste lines
- hot and cold water
- exhaust ventilation
- steam—high and low pressure
- electricity for illumination and power
- heating
- air conditioning
- automatic fire sprinklers

Subdivisions of the Blood Bank

The complete blood bank should have the following physical areas: waiting room, donor interviewing area, examining area, bleeding area, commissary, laboratory, blood and blood product storage area, storage area for equipment, glassware and reagents.

How to Determine Total Size of the Blood Bank

No matter what the source of its blood supply, laboratory facilities are required. To determine the size of this area, it is necessary to estimate the number of anticipated laboratory procedures and the number of personnel required to perform them. In well organized departments carrying out recommended approved procedures, it can be estimated that each pint of blood prior to transfusion requires at least 13 to 14 different tests. This number may be increased if the blood is not given to the anticipated recipient but is to be set up again for another recipient. These tests include:

Donor: 6 + tests
- ABO typing
- A & B back typing (confirmation)
- CDE typing plus D^u if negative
- Testing with pooled O cells for atypical antibodies
- Determination of titer of iso-agglutinins A & B in type O
- Serology

Recipient: 4 tests
- ABO typing
- D typing plus D^u if negative
- A & B back typing
- Testing with pooled O cells for atypical antibodies

Cross-Match: 3 tests
- Saline crossmatch
- Protein crossmatch
- Saline Coombs

This list does not include special procedures in which studies for other blood types or antibodies are carried out. In such situations panel cells and trypsinized cells are used or elution or inhibition procedures are performed. Thus, each 100 units of blood would require approximately 1,300 tests. If 100 units of blood were transfused each month, approximately 15,000 tests per year would be accomplished.

From previous studies[2] it was shown that approximately an average of 45 tests per square foot of laboratory area can be performed, that an average, well trained medical technologist can carry out approximately 12,000 to 15,000 tests per year and that one technologist requires about 350 gross square feet.

Thus, 15,000 tests would require approximately 330 square feet gross. This is in close agreement with space requirements necessary for a technologist to carry out these procedures.

The laboratory should be arranged in the form of a module. A module may be considered as the work space determined by actual needs. The individual laboratory module has within it all the facilities necessary for a complete room. It possesses natural and artificial illumination; heat, ventilation and air conditioning;

pipes for all major services and reserve space for future and special wants; required electrical connections and fixtures; necessary sinks and drainage; access from a common corridor and egress to another contiguous unoccupied space for safety and the required amount of floor area for counters and aisles.

The counters are usually extended at right angles from either the outside or inside walls. It is recommended that the width of the module be ten feet from center to center of the peninsulas with an aisle of five feet between them and with counters approximately 22-24 inches wide.

It is not our intent to describe the laboratory floor plan, finishes, sound-proofing, flooring, etc., in detail. Such matters are described in "Manual for Laboratory Planning and Design" published by the College of American Pathologists.[2]

Hospital wings may be of two widths—either 42 or 54 feet—with a central eight foot corridor. The rooms on each side of this corridor may be approximately 16 feet long or 20 feet long. Thus modules either 10 × 16 or 10 × 21 feet would be created. In such instances, the laboratory area should consist of two modules (320 square feet) in the narrow wing or one and one-half modules (300 square feet) in the wide wing, to meet the requirements of the hypothetical case described above. Multiple repetitive modules may be arranged back to back, depending upon the total number of procedures to be performed.

In the narrow wing, the peninsulas should be approximately ten feet long. Thus, with four counters, one would have approximately 40 linear feet of counter space. In the wide wing there would be three counters about 15 feet long each and thus there would be 45 linear feet of counter surface. This arrangement should certainly suffice for the performance of 15,000 laboratory procedures per year. If the peninsulas extend inward from the outside wall, there should be open areas along the corridor wall where refrigerators, incubators, and waterbaths may be placed.

Bleeding Room

Whether or not the blood bank bleeds donors at present, provisions for construction of such a room should be made at time of planning. The size of this can be estimated from studies reported by Davidsohn and Stern[1] based on a questionnaire sent to a large number of blood banks throughout the country. The basic question is to determine the number and size of donor tables to be installed. Over 50 per cent of the responses suggested tables 72 or 74 inches long and 23 to 26 inches wide.

These authors calculated the work load per donor table. They showed that 40 per cent of the banks draw 50-100 units per bed per month and 20 per cent of the banks could draw from 100-150 units per bed per month. Thus, in three-fifths of all banks, 50-150 units could be obtained per bed per month.

As for donors accommodated per bed, 38 per cent of the banks reported two to three donors per bed per hour, 38 per cent three to four donors per bed per hour and 12 per cent four to five donors per bed per hour. Eighty-five per cent of the banks considered five to ten minutes the optimum time for drawing 500 cc. of blood. Seventy-five per cent of the respondents suggested that the time of rest should be between five and 15 minutes. A reasonable number would be three to four donors per bed per hour and each bleeding would require approximately 15 to 20 minutes.

In a blood bank drawing 100 units a month, one donor table would certainly suffice. However, two donor tables should be available to reduce waiting time and to permit two bleedings to take place simultaneously. A module ten feet wide would suffice for two donor tables. These may be arranged parallel to each other and leave an aisle of four or five feet between the beds which would suffice for easy access and for the installation of stands and other equipment. The two beds may also be arranged along one partition, head to head, with cabinets or counters on the opposing wall for storage of bottles, tourniquets, reaction trays, etc.

Although the use of donor tables is the classical arrangement of procuring blood, it should be emphasized that recently bleeding chairs, tilting chairs and other arrangements are being introduced which may eliminate the need for tables and thus lead to revision of these space requirements.

A waiting room, receptionist or clerk area, is required. A module of 10 × 20 feet could accommodate these functions with about one-third devoted to waiting room and two-thirds to the clerical area. In the latter zone interviews and history taking could be accomplished.

Storage of supplies including glassware, tubing, fluids, etc. and storage of the blood supplies within the necessary refrigerators could be placed in an additional module, similarly ten feet wide. These two areas could be separated by a partition and, if properly designed with

sufficient cabinets and shelving, would furnish sufficient space for an alcove in which commissary functions could take place. On the basis of the above estimates it would appear that the minimum area required for the blood bank in the hypothetical case stated above, would comprise between 800 to 1,000 square feet.

A complete description of the physical layout of partitions is not considered within the framework of this paper, inasmuch as so many variables exist as to the structure of the building, location of columns, pipe spaces, stairways and elevator shafts. Many of these questions are completely discussed in the "Manual for Laboratory Planning and Design"; the reader is directed to that source for further information.

Summary

1. Various factors entering into the estimates of necessary space for blood bank operations are described.

2. Certain mathematic calculations are given for estimating size of laboratory, bleeding room and other areas.

3. Necessity of planning for growth, expansion or change is stressed.

4. Opinions are voiced concerning location of blood bank in the hospital.

References

1. Davidsohn, I. and K. Stern: A Survey of Blood Bank Practices Related to Donors and Withdrawal of Blood, presented at the Fourth Annual Convention of the American Association of Blood Banks, October 22-24, 1951, Minneapolis, Minn.

2. Rappoport, A. E.: Manual for Laboratory Planning and Design, published by The College of American Pathologists, Chicago, Ill., 1960.

Executive Office of the President
Office of Civil and Defense Mobilization

Washington 25, D. C.

National Blood Program

Statement of Basic Principles

An effective blood program is of major national importance. It is particularly so now because of world tensions. Programs designed to provide in an emergency sufficient blood and related items and activities to meet basic civilian, military and civil defense needs have been developed by the American National Red Cross, other civilian blood banking systems, the Department of Defense and the Office of Civil and Defense Mobilization.

Coordination of these programs is essential in order to meet the emergency blood needs of the nation without unnecessary duplication of effort or conflict of interests. This can best be achieved by integrating the related blood programs operating or cooperating on a national scale into a single national blood program. A program of this type is of prime importance to the Federal agencies directly involved and to civilian organizations engaged in blood programs, such as the Joint Blood Council and its member institutions. These are the American Association of Blood Banks, American Hospital Association, American Medical Association, American National Red Cross and American Society of Clinical Pathologists.

Pursuant to the President's letter of December 10, 1951, and The National Plan for Civil Defense and Defense Mobilization promulgated by the President in October 1958, the following principles are outlined:

A. General

1. The program created to meet the nation's needs for blood and related items and activities during the national emergency shall be called "The National Blood Program."

2. The National Blood Program represents a coordination of the blood programs now existing or which may be developed to meet a national emergency.

3. The Office of Civil and Defense Mobilization will exercise authoritative coordination, including development and promulgation of over-

all policy guidance of the National Blood Program. The Office of Civil and Defense Mobilization will adjudicate conflicts between participating Federal agencies. A Committee on Blood is established within the organization of the Health Resources Advisory Committee to advise and assist the Director, OCDM, in the discharge of these responsibilities.

4. The Department of Health, Education, and Welfare has been delegated operational responsibility for the nonmilitary aspects of the National Blood Program as part of its assignment of responsibility for the nation's health mobilization.

5. No agency of the Federal Government participating in the National Blood Program shall duplicate the efforts of another agency except in situations where it is clearly recognized that the task in question cannot be otherwise adequately performed. Further, any such duplicating effort shall not be undertaken without prior agreement among the Federal Agencies involved and the Office of Civil and Defense Mobilization. All other agencies participating in the program are urged to coordinate their efforts with all other participants so as to avoid unnecessary duplication.

6. Separate reserves of blood, blood derivatives, plasma expanders and related items shall be established and maintained by the Department of Defense and by the Department of Health, Education, and Welfare under assignment of responsibility by the OCDM.

B. Recruitment of Volunteer Blood Donors

When directed by the Office of Civil and Defense Mobilization, the total donor recruitment program shall be geared to soliciting donors for the National Blood Program as a whole rather than for specific parts of the whole. The various agencies involved in the National Blood Program shall unite in a coordinated program of public information in order to inform the people clearly of the urgent need for blood.

C. Blood Collection Activities

1. The American National Red Cross is designated as the blood collecting agency for Federal agencies participating in the National Blood Program and shall cooperate with other member institutions of the Joint Blood Council in carrying out this designated responsibility.

2. The collection facilities which the Department of Defense has established or proposes to establish shall be limited to Armed Services installations and blood shall be drawn only from military personnel or from civilian personnel on military installations.

3. Blood derivative reserves for the National Blood Program shall be established by contracting with the American National Red Cross for the collection of blood for this purpose, except that the Department of Defense may utilize blood salvaged from the Department of Defense Blood Collection Program for the purpose of adding to the Department of Defense blood derivative reserve.

D. Emergency Allocation of Blood and Blood Derivatives

1. The Director, Office of Civil and Defense Mobilization may, in an emergency, allocate blood collected by organizations actively participating in the National Blood Program. With modifications dependent on the magnitude and type of emergency, the following priorities will be applied:

(a) First priority shall be given to the allocation of blood to the Armed Services for whole blood transfusion purposes.

(b) Second priority shall be given to the allocation of whole blood for civilian needs.

(c) Blood or blood derivatives going into the reserves shall be allocated according to military and non-military defense requirements to the Department of Defense and the Department of Health, Education, and Welfare by the Director, Office of Civil and Defense Mobilization.

2. As and when directed by the Office of Civil and Defense Mobilization, the Department of Defense and Department of Health, Education, and Welfare shall provide the Office of Civil and Defense Mobilization with reports covering (1) the respective requirements and reserves of blood program items and (2) related activities.

3. In the event of enemy action, the total reserves of blood, blood derivatives and plasma expanders shall be subject to immediate reallocation by Executive Order.

E. Research on Blood and Related Problems

1. The Interdepartmental Committee on National Blood Program Research established by the Department of Defense shall be maintained. The primary mission of the Committee will be to coordinate Federal agency funding and programming aspects for research and development projects relating to the National Blood Program so as to best support that program. How-

ever, this mission should not be construed as control or direction of the research of any agency represented on this committee.

2. A Committee on Blood and Related Problems shall be maintained by the National Research Council to:

(a) Formulate, evaluate and recommend programs and projects relating primarily to scientific aspects of the National Blood Program.

(b) Recommend actions which may be taken by the various agencies involved in the operation of the National Blood Program based on relevant research determinations.

3. Funds shall be provided for the work of the National Research Council Committee on Blood and Related Problems by participating agencies in accordance with established practices.

4. Interested Federal agencies, such as the Department of Defense, American National Red Cross, Office of Civil and Defense Mobilization, Public Health Service, and the Food and Drug Administration, shall seek the advice of the National Research Council Committee on Blood and Related Problems on problems relating primarily to the scientific aspects of Research and Development for the National Blood Program.

5. The National Research Council and the Department of Defense shall keep each other and the Office of Civil and Defense Mobilization informed of the progress of the respective committees. Reports of the activities of the two committees shall be submitted when and as requested by Office of Civil and Defense Mobilization. November 9, 1960

The Statement of Basic Principles dated June 3, 1957, is hereby rescinded. (OCDM-DC 33674)

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Book Reviews and Abstracts

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Reviews

Syllabus of Laboratory Examinations in Clinical Diagnosis. Lot B. Page and Perry J. Culver, Cambridge, Mass., Harvard University Press, 1960, 580 pp., 98 tables, 56 figures. $12.50.

Soon after its publication in 1950, Thomas Hale Ham's *Syllabus of Laboratory Examinations in Clinical Diagnosis* became a standard textbook in many medical schools and clinical laboratories. It had been written largely by members of the faculty of the Harvard Medical School for students in that school. It was a scholarly and painstaking piece of work. Thousands of students were fortunate to have had it as their guide in the learning of laboratory medicine.

A revised edition of the Syllabus has now appeared, ten years after the first. Having left the Harvard Medical School, Dr. Ham's authorship has been taken over by two other physicians. The contributors are also largely from the same faculty. Both the contributors and the critics represent almost an entirely new group. Since these authors are new to the Syllabus, the material has been virtually rewritten. The format has been considerably improved by a much more readable presentation of the material, by the use of conventional printing rather than varitype and by a hard cover. Of course, the original low price has disappeared.

The material has again been presented in distinct units—each written by one or more contributors. There are now 34 units in place of the original 27 and 84 pages have been added. Additional material includes cardiac catheterization, pulmonary function tests, several units on metabolic diseases, the LE cell test, plasma proteins, electrophoresis and much other data that has become part of medical laboratory practice during the past ten years. Tests that the physician or house officer might be called on to perform are described in detail (as before). For the more specialized tests, principles, interpretations and limitations are described but the procedural details are omitted. Greater emphasis has been placed on the pathologic physiology of diseases and on principles underlying the various tests and measurements.

Typical of the new edition is the change that has been made in the chapter on blood groups. The author is new, the material is similar, though a few recently discovered items have been added and the presentation is quite different—thus reflecting the new author's interpretation of the relative importance of things. This chapter is certainly more readable and, I should surmise, more easily mastered by the student.

The revised edition of the Syllabus is a new book that replaces and up-dates a popular old favorite. The contributors and editors have continued their policy of fitting laboratory data into its proper perspective in the total care of the patient. The revised edition is an excellent improvement over the original one and the popularity of the *Syllabus* should continue unabated.—*John B. Ross.*

The Chemistry of Heredity. Stephen Zamenhof. Springfield, Ill., Charles C Thomas, 106 pp.

An Outline of Chemical Genetics. Bernard S. Strauss. Philadelphia, Pa., W. B. Saunders, 188 pp.

Biochemistry of Human Genetics. A Symposium edited for the CIBA Foundation by G. E. W. Wolstenholme and C. M. O'Connor. Boston, Mass., Little, Brown & Co., 340 pp.

THERE are few studies in the life sciences that have evoked as much interest as have the biochemical aspects of genetics. The events of the past five years have led to an imposing list of publications in this area, with a promise of still greater things to come. Scarcely a field in medicine is not touched by these startling developments. Workers in blood banking, transfusions and the transplantation of organs have a large stake in this trend and are, therefore, obliged to keep up with these rapidly mounting advances. Three of the better recent books on biochemical genetics are simultaneously reviewed herein.

The first two are small volumes that were written to present some of the fundamental aspects of biochemical genetics, to an audience that requires some basic grounding in this area. Both Zamenhof and Strauss assume in the reader, a basic knowledge of biochemistry as well as the laws of Mendelian hereditary.

Zamenhof's work is very short. Its 106 pages are divided into seven chapters entitled, Mechanisms of Heredity; Heredity Determinants as Chemical Substances; The Nucleic Acids; Mutation; Intermediary System and Cytoplasmic Inheritance; and The Peripheral Reactions. There is a limited clinical application in that the final chapter (8 pages) is devoted to Hereditary Defects in Man. This little volume presents a concise and very lucid summary of what is known of the chemical basis of heredity. One of its chief virtues, apart from its brevity, is the highly personal and intimate style employed by the author, without sacrificing its scientific or technical correctness. This little book is strongly recommended as must reading to anyone who desires an introduction to this subject.

Strauss' book is the product of a series of lectures delivered to the Faculty of Medicine at Osaka University. It too presents the basic aspects of biochemical genetics in an interesting and personal manner. It has a greater tendency to ramble than the former book but presents its material in a more detailed and documentary fashion. This is probably due to its basic organization as lecture material.

Its six chapters deal with The Genetic Control of Protein Synthesis; The Chemical Nature of the Hereditary Material; The Molecular Nucleo-Cytoplasmic Relationships and the Problem of Protein Synthesis and the Biochemical Genetics of Man. As in the former book, the basis of chemical genetics are treated lucidly, and even oversimplified wherever possible. Application of basic genetics to clinical problems is employed in order to illustrate one of the author's points. This book should also be required reading for anyone who desires to learn fundamentals of biochemical genetics.

The CIBA Foundation volume is the result of a symposium on human genetics held in Naples, Italy in May 1959. It is exemplary of the excellence achieved by these series and as in others is characterized by an informal, conversational type of presentation. The discussion that follows each topic is stimulating, lively and highly informative.

The chapters most likely to be of interest to readers of this periodical include Genetical Analysis in Man (Penrose); The Genetics of Primaquine Sensitivity of Erythrocytes (Childs and Zinkham); Chemical and Genetical Units of the Hemoglobin Molecule (Itano, Singer and Robinson); and the Abnormal Human Hemoglobins (Hunt and Ingram).

Short but important communications deal with fetal myoglobin, genetics of the plasma protein variants, gamma globulins, haptoglobins and transferrins, immunochemical aspects of the products of blood group genes, and the biosynthesis of blood group substances. The articles by Morgan, Watkins and Ceppellini, which deal with the specific mucopolysaccharide structure of blood group substances will be very well received by immunologists and hematologists. Each of these three books represents a significant achievement.—*Anthony V. Pisciotta, M.D.*

The Nation's Blood Transfusion Facilities and Services. Joint Blood Council, 1832 M Street, N.W., Washington, D. C.

THIS ANALYSIS of data regarding the nation's transfusion facilities and services was compiled from the information contained in the Directory of Blood Transfusion Facilities and Services prepared by the Joint Blood Council. Although the information is based on a 1956 survey, it is not likely that the general picture has changed markedly during the last few years. In the summary, Dr. Leroy E. Bates, President of the Joint Blood Council, suggests the need for data on phases of transfusion services, particularly in the realm of donor recruitment,

staffing patterns and criteria for administering blood and derivatives.

The survey reveals that the nation's blood needs are supplied about equally by the Red Cross Centers and by the community and hospital blood banks. It is believed that the report covers at least three-quarters of all the blood handled in the United States. Among the interesting observations was the variation in blood banks and blood usage according to the geographical location of the institution. For example, in New England, 70 per cent of the blood collected was drawn by the Red Cross and the remaining 30 per cent by hospitals. On the other hand, community and hospital blood banks were about equally responsible for blood collection in the West South Central Region. Hospital blood banks depended upon other sources to supplement their supply of blood since they were able to collect only one unit for every 1.8 units transfused by them. Medium-sized hospitals reported a greater usage of blood per bed than larger or smaller institutions. The ratio of blood given per hospital bed also varied with the location of the hospital.

The pamphlet contains useful statistics for individuals interested in blood banks both from the standpoint of planning future institutions as well as the operation of current installations. The proposed ideas for future surveys are worthy of consideration and would be of value. —*L.R.G.*

Abstracts

Blood Groups and Antibodies

Genetic influence in iso-immunization in the rabbit. C. Cohen. Battelle Memorial Institute, Columbus, Ohio. J. Immunology. 85: 144, 1960.

THE BLOOD groups of rabbits fall into five genetic systems. Iso-immunization using only incompatibility to the antigens in one of these systems (Hg.ADF) does not result in uniform iso-antibody production. The results suggest that certain combinations of donor and recipient blood groups have a higher probability of inducing iso-immunization than do others. Antibody productivity appears to be the result of the balance between compatible and incompatible antigens. Thus, in humans, cDE more readily immunizes the female cde than does CDe and the resultant erythroblastosis is more severe. Similarly, CDe often provokes the production of anti-C in addition to anti-D in iso-immunized women, while anti-E is only rarely found in association with anti-D in cde women stimulated by the antigen cDE.—*A. S.*

(The author points out that the evidence for higher liability for isoimmunization of Rh negative women with Rh_2 husbands and greater severity of erythroblastosis in Rh_2 infants is not conclusive. See Kirk, R. L., *et al.*, Brit. J. Haemat. 5: 158, 1959.—Ed.)

The destruction of red cells by antibodies in man. III Quantitative factors influencing the patterns of hemolysis *in vivo*. J. H. Jandl and M. E. Kaplan. Boston City Hosp., Boston, Massachusetts. J. Clin. Invest. 39: 1145, 1960.

THE INFLUENCE of antibody/antigen proportions on the sites and kinetics of red cell destruction were studied in normal and agammaglobulinemic human subjects, and also in rats, employing a rabbit antibody against rat red cells. Hepatic uptake of antibody-injured erythrocytes was not pronounced until agglutinating amounts of antibody were employed, although some hepatic sequestration was observed at antibody concentrations producing little or no agglutination *in vitro*. The evidence suggests that the liver may partially participate in red cell trapping in a "spleen-like" fashion both in splenectomized subjects and also in the presence of a vast excess of incomplete antibody. Purely hepatic erythrocyte destruction patterns, however, are associated with demonstrable red cell agglutination *in vitro*.

Intravascular hemolysis by agglutinating antibodies occurred only with very large doses of antibody.—*H. F.*

Serology and genetics of a new blood factor: hrs. M. Shapiro. South African Blood Transf. Service, Johannesburg, South Africa. J. Forensic Med. 7: 96, 1960.

The ninth pregnancy of a Bantu woman terminated in the delivery of an erythroblastotic infant. The child was typed A rh and the direct Coombs test was strongly positive. The mother was typed O rh. The mother's antibody agglutinated all the cells of a comprehensive panel. Later testing showed that both mother and child were a very low grade Rh_o.

Blood grouping tests showed that the reactions were enhanced by enzymes and optimal agglutination occurred at 37 C. Other tests seemed to eliminate all the known high-frequency antibodies. Comparative studies with cells and sera from a negress and Porto Rican woman in other parts of the world focused attention on unusual hr" reactions.

The Bantu woman's serum contained two antibodies, designated anti-Hr and anti-hr^s. The anti-Hr component appeared to be similar to the antibody formed by Rh_o (–D–/–D–) individuals. The second antibody reacted like anti-hr″. Anti-hr^s is contained in most, if not in all, sera hitherto regarded as anti-hr″; some anti-hr″ sera contain only anti-hr^s. An hr″ positive person may produce anti-hr^s. Thus anti-hr^s is related to hr″ as anti-Rh^A, anti-Rh^B, etc. are related to Rh_o.

Using the original serum and preparations derived from commercial anti-hr″ sera, extensive population and family studies were undertaken to define the incidence and inheritance of the hr^s factor. From a forensic point of view, recognition of this blood factor explains certain anomalous findings previously attributed to illegitimacy or to "chromosome deletions."
J. B. R.

(A careful reading of the original article is essential to understand this subject.—Ed.)

Amniocentesis in Rh-immunized mothers. E. Mackay. Royal Women's Hosp., Melbourne, Australia. Med. J. Australia. 2: 474, 1960.

EARLIER results reported by Bevis and by Walker indicated that amniotic fluid could furnish useful information regarding the prognosis of babies born to Rh-immunized mothers. Amniocentesis (puncture of the amniotic sac) was performed by inserting under local anesthesia a 22-gauge needle into the midline of the abdomen at about the thirty-second week of pregnancy. The amniotic fluid was examined spectrophotometrically for its content of oxyhemoglobin and bilirubin pigments. In this series a sample of amniotic fluid taken at delivery was also examined and results compared to the earlier sample.

It is believed by the author and by previous workers that this procedure is sometimes more valuable than other better established technics in indicating the optimum time for premature delivery of erythroblastotic infants.—*J. B. R.*

Sensitization to rare Rh Antigen V. J. Geiger (Lenox Hill Hosp., New York, N. Y.). J.A.M.A. 173: 1590, 1960.

THE AUTHOR reports finding anti-V (designated anti-rh^v by Wiener) in the serum of a patient who had received at least 25 transfusions. This rare antibody was detected by screening the recipient's blood with a pool of Group O cells possessing numerous blood factors including V. This screening technic is employed by the hospital blood bank to test both donors' and recipients' sera and is used in addition to the routine cross-matching procedures.—*P. M.*

Anti-A Haemolysin in group O blood donors. An East Anglian survey. C. B. V. Walker and H. G. Dennis (Regional Blood Transfusion Centre, Cambridge, England). Brit. Med. J. 2: 1303, 1959.

RESULTS of a screening test for anti-A hemolysin in group O donors, using horse serum as a source of complement, are described. There was a tendency to encounter anti-A hemolysin with increasing frequency as the ages of the donors rose. Armed services personnel did not have significantly greater incidence of hemolysins despite their high frequency of vaccination and similar innoculations. In the entire series of 25,541 consecutive group O donors, 2,078 (8.1 per cent) gave a positive result.—*H. A. P.*

Anti-M antibody as a rare cause of haemolytic disease of the new-born. R. T. Simmons, V. I. Krieger and R. Jakobowicz. Commonwealth Serum Laboratories, Melbourne, Australia. Med. J. Australia. 11: 336, 1960.

THREE successive babies of blood type MN in one family suffered from hemolytic disease of the new-born when the only atypical antibody demonstrable was a low-titer placenta-permeable anti-M agglutinating antibody, most active at a low temperature range. In each case the direct Coombs test was negative but free anti-M could be demonstrated in the cord serum. The maternal antibody was also at times demonstrable at 37 C. in low titer.

This example of anti-M was but one of at least 20 that the authors have encountered in the past ten years. Anti-M is, however, a very rare cause of hemolytic disease of the newborn.—*J. B. R.*

Blood-group antibodies and red-cell destruction. P. L. Mollison. Postgraduate Medical School, London, England. Brit. Med. J. 2: 1035 and 1123, 1959.

THIS IS an excellent review of red cell antibodies, emphasizing their patterns of reactions with red cells both *in vivo* and *in vitro*. The data are much too voluminous to abstract, and anyone interested in red cell antibodies, blood bank laboratory procedures or transfusion therapy is strongly urged to read the original papers. —*H. A. P.*

Effect of ribonuclease on erythrocyte antigens. E. Hackel and R. E. Smolker. Michigan State Univ., East Lansing, Michigan. Nature. 187: 1036, 1960.

RED CELLS were first exposed to the enzyme ribonuclease. They were then washed and titrated with various isoagglutinins (saline agglutinins). With anti-Rh and anti-Lutheran antibodies the agglutinability of the treated cells was much less than that of untreated controls. It seems reasonable to conclude that the affected cells lost ribonucleic acid components. This and other evidence suggests that Rh and Lutheran antigens contain ribonucleotide components.—*J. B. R.*

Specific inhibition of Rh_o (D) antibody by sialic acids. Matthew C. Dodd, Nancy J. Bigley and Virginia B. Geyer. Ohio State University, Columbus, Ohio. Science. 132: 1398-1399, 1960.

CRUDE and crystalline preparations of N-acetyl neuraminic acid were found to inhibit maximal agglutination of anti-D. N-glycolylneuraminic acid, a beef brain ganglioside and a polysaccharide from a species of Pseudomonas were also effective inhibitors. The inhibition was specific for anti-D—no inhibition being observed with anti-C-E-c-e. In this preliminary report it is not completely clear what concentrations of the materials were used. *p*H was controlled.—*T. J. G.*

Storage and Transfusion

Viability of red cells of ACD blood aseptically separated from plasma in a closed plastic system. J. G. Gibson, II, J. C. Pond and T. J. McManus (Harvard Med. School, Boston, Massachusetts). New Eng. J. Med. 263: 634, 1960.

Method of separation	% plasma recovered	% post-infusion survival of red cells after 21 days' storage
Sedimented for 24 hrs at 5 C.	48	83
Centrifuged 10 min. at 500g, room temperature	88	81
Centrifuged 10 min. at 250g at 5 C.	70	86

A PLASTIC blood collection unit, in which a second bag is attached to the first by a length of tubing, provides a completely sterile unit that permits separation of plasma from packed cells at any time during storage without fear of bacterial contamination. Red cells were separated from plasma within 24 hours of collection by three different technics and were then stored at 4 C. for 21 days prior to administration. For results see table.

It is concluded that all methods provide red cells with viability comparable to that observed in stored whole blood.—*H. A. P.*

Edglugate-MG as a blood preservative for extracorporeal circulation. C. F. Kittle and R. Eilers (Univ. Kansas Med. Center, Kansas City, Kansas). Arch. Surg. 81: 179, 1960.

BLOOD was collected and stored for as long as four days in the EDTA anticoagulant proposed by Smith and Brown. Heparin and then calcium were added prior to filling the pump-oxygenator. Experiences with 69 open heart surgery cases and 107 routine transfusions were encouraging, and the authors believe that they warrant continued use of this anticoagulant-preservative solution. In view of the relatively rapid rise of plasma potassium on storage of blood collected in this manner, caution must be exercised to avoid administration of too much potassium to small children or patients in uremia.—*H. A. P.*

The preservation of blood for transfusion. IV *in vitro* recovery after freezing and thawing of red cells modified with sugars. V Post-transfusion survival of red cells modified with sugars, frozen and stored in the frozen state. M. M. Strumia, L. S. Colwell and P. V. Strumia. Bryn Mawr Hosp., Bryn Mawr, Pa. J. Lab. & Clin. Med. 56: 576 and 587, 1960.

THE protective action of dextrose and lactose on red cells during freezing of whole blood was investigated and optimum concentrations established. *In vitro* recovery of red cells after freezing and thawing was better the more the blood was diluted. Results were superior when freezing and thawing was rapid and with agitation. Under optimal conditions 95 ± 3 per cent of the red cell population was recovered intact.

The survival of stored frozen red cells was measured by chromium labeling technics following transfusion. The 24 hour survival averaged 88 per cent as compared with a value for

fresh normal cells of 94 per cent. Survival in the period subsequent to 24 hours paralleled that of fresh cells. When blood was stored at −18 C., recovery and survival of red cells decreased sharply. At −70 C. the decrease was less rapid but still unsatisfactory. At −93 C. there was no loss due to storage alone for as long as two years. Combining the loss of red cells during *in vitro* manipulation and those lost *in vivo* in the first 24 hours, the total effective recovery plus survival was 77 per cent.—*H. A. P.*

The effect of resuspension and shipment on the viability of frozen red cells. P. J. Schmidt and J. L. Steinfeld. National Institutes of Health, Bethesda 14, Maryland. Clin. Science. 19: 109, 1960.

THE authors describe a method of storing at 4 C. red blood cells previously frozen in glycerol. This greatly increases the practicality of utilizing such cells. All procedures were carried out aseptically from donation to transfusion using a single plastic bag specially designed with 12 ports.

Best results were obtained when thawed red cells were suspended and stored in a citrate-dextrose medium containing phosphate, magnesium and potassium ions. Addition of albumin to this solution provided no additional benefit. Red cells could be stored in this medium for longer than ten days without significantly affecting their subsequent *in vivo* survival. They could be transported to a distant site without effect.

The over-all results showed that 10 per cent of the cells were lost in processing. The frozen red cells could be stored up to five years before the number of viable cells dropped below 70 per cent. The thawed cells suspended in the above medium for periods up to ten days had an *in vivo* survival of over 85 per cent.—*H. A. P.*

The use of citrated banked blood for open-heart surgery. N. Zuhdi, B. McCullough, J. Carey and A. Greer. 430 Northwest 12th Street, Oklahoma City 3, Oklahoma. Anesthesiology 21: 496, 1960.

A TECHNIC is described which avoids the use of heparinized donor blood in open-heart surgery. A bubble oxygenator with double helix and small priming volume is filled with 5 per cent dextrose in water. The only donor blood used is ACD blood transfused directly into the patient to cover blood loss at surgery.

Recirculation of ACD bank blood through the pump-oxygenator for 60 minutes without connection to a patient resulted in only minor fall in hematocrit, white cell and platelet counts, with insignificant rises in plasma hemoglobin and potassium, if the blood had been stored five days or less.

This technic was used in 30 patients who had open-heart surgery. The average fall in hematocrit was only 2.3 per cent; the average rise in plasma hemoglobin was 18.9 mgm. per cent. Platelet counts were not reported. Only two to three units of ACD blood were used in most of the cases which were reported in detail. The maximum number of units of ACD blood administered to one patient was nine units, during a bypass that lasted 181 minutes. Citrate intoxication was not noted.

Safety of the technic with pump-oxygenators requiring larger priming volume has not been determined. (It is of interest that rapid mixing of patient blood with the dextrose solution had no apparent harmful effect, despite the known tendency of dextrose to clump red cells.)—*H. A. P.*

(At the 1960 meeting of the American College of Surgeons, Foote and Maloney reported that ACD blood can be heparinized, recalcified and used to prime a pump-oxygenator. The safety of these new procedures needs to be further explored before recommending their adoption for routine use.—Ed.)

Letter to the Editor: Mortality due to blood transfusion. J. H. Joseph. Lancet. 2: 709, 1960.

BY REFERRING to vital statitiscs collected in both England and the United States in recent years (1954-1958), it is concluded that deaths from blood transfusions are relatively rare today, estimated to be one in 250,000 to 350,000 transfusions. These figures are in sharp contrast to the one death in 1,000-3,000 transfusions reported by other authors in the period up to 1952.—*J. B. R.*

(The reliability of "vital statistics" in relation to transfusion mortality may be questioned. Certainly only a fraction of the hemolytic and overload reactions, accidents involving air embolism and instances where less dramatic consequences of transfusions are an important, if only contributory, cause of death are detected and recorded.—Ed.)

Coagulation

Plasma clotting factors in chronic hepatocellular disease. S. I. Rapaport, S. B. Ames, S. Mikkelsen and J. R. Goodman. From the Department of Medicine, University of

Southern California School of Medicine and University of California Medical Center at Los Angeles and the Long Beach Veterans Administration Hospital, Long Beach, California. New Eng. J. Med. 263: 278, 1960.

A COMPARISON of prothrombin times with more specific clotting factor assays was made in a study of patients with chronic hepatocellular disease. In patients with prothrombin times in the 15-30 per cent range by either the Quick or the prothrombin-proconvertin method, deficiencies were always noted in prothrombin, proconvertin, and proaccelerin and possibly in Stuart factor and plasma thromboplastin component. In patients with moderately prolonged prothrombin times, the tests were not reliable indicators of proaccelerin and proconvertin levels. Deficiencies in plasma clotting factors other than prothrombin might be severe enough to result in abnormal bleeding. Thrombocytopenia has also been described in cirrhotic patients.

These workers feel that the development of procedures for preparing effective plasma clotting factor concentrates and stored, viable platelet concentrates will reduce the mortality rate due to uncontrollable hemorrhage in patients with cirrhosis. Until such preparations are available fresh blood less than four hours old collected plastic bags or silicone-coated bottles is recommended for cirrhotic patients with thrombocytopenia. For other cirrhotic patients blood less than seven days old should be transfused since most of the proaccelerin activity would be retained up to that time.—*L. R. G.*

Plasma therapy in haemophilia. B. J. Arnold and W. R. Pitney. Royal Perth Hosp., Perth, Western Australia. Med. J. Australia. 2: 660, 1960.

FRESH or fresh frozen plasma was used in the treatment of nine hemophiliacs during 12 episodes of actual or threatened hemorrhage, including spontaneous hemorrhage, dental extraction, surgical procedures and traumatic events. For intelligent management, laboratory control was considered essential; the most useful test was the thromboplastin screening test of Hicks and Pitney (Brit. J. Haemat. 3: 227, 1957), which may be performed with rapidity and is simpler to execute than a thromboplastin assay.

The authors aimed to achieve a satisfactory plasma anti-hemophilic factor concentration—35 per cent of that found in normal blood—as rapidly as possible, and to maintain an adequate concentration until bleeding had ceased. Patients were therefore given an initial transfusion of one liter of plasma in an hour or less. Subsequent transfusions depending on the results of laboratory tests, were usually given at the rate of 250 ml. every four hours. The total number of 250 ml. units employed ranged from three to 52 and averaged 15 per bleeding episode.—*J. B. R.*

The concentration of antihaemophilic globulin (AHG) related to age. A. A. Cooperberg and J. Teitelbaum. Jewish Gen. Hosp., Montreal, Canada. Brit. J. Haemat. 6: 281, 1960.

THE concentrations of antihemophilic globulin (AHG) was assayed in a group of individuals aged 17-78 years. With advancing years there was a steady rise in the average AHG level, especially in those over 40, until the sixth decade. The mean concentration in males was higher than in females.

The authors suggest that blood or plasma of the older male population be used for the treatment of patients with hemophilia.—*J. B. R.*

The viability of stored human platelets. M. Baldini, N. Costea and W. Dameshek. New Eng. Center Hosp., Boston, Massachusetts. Blood 16: 1669, 1960.

BLOOD was withdrawn by gravity into plastic bags using EDTA as anticoagulant. The platelets were separated by differential centrifugation and labeled with radioactive sodium chromate. After varying periods of storage in different media, the tagged platelets were reinfused into the original donors.

As criteria of viability, survival time and maximum percentage of platelet radioactivity were measured. From these two values, an index of viability was calculated and expressed as a per cent of the value obtained with freshly prepared platelets.

An inverse relationship between time of storage at 4 C. and platelet viability was found. Thus after three hours of storage, the viability index was reduced to 62 per cent, at 24 hours to 12 per cent and at 48 hours to 2 per cent. No significant difference was observed whether the platelets were suspended in whole blood, platelet-rich plasma or as concentrates in plasma. When stored in saline, gelatin or in glycerol after freezing viability was further reduced.

A practical conclusion to be drawn is that platelets for transfusion should be infused within less than three hours after collection.—*J. B. R.*

Questions and Answers

Donor Standards

Q. "The problem of history of malaria in a donor has raised the question of how long after a clinical attack or use of antimalarial drugs does a person become acceptable for blood donations. The American Red Cross uses two years on their history card and since we are members of their blood program we have been using the same. However, Mollison has reported an instance of malaria being transmitted in this fashion twenty years after clinical illness in a donor not on suppressive therapy since; a few other cases are said to be on record similarly.

Because of obvious medico-legal as well as public health considerations we are anxious to solve this question and would appreciate any recommendations you might have." — *M.D., Schenectady, New York.*

A. "The problem you raise has been thoroughly considered by the Blood Banks Committee of the Massachusetts Medical Society and the substance of that consideration has been incorporated into the State Transfusion Regulations.

This requires the permanent rejection of blood donors with a history of malaria. In addition, persons who have lived in a malarious area without contracting the disease are acceptable as donors providing they have lived outside of the malarious area for one year without manifesting the disease regardless of the previous use or non-use of anti-malarial drugs.

The American Red Cross regulation with regard to a free two year interval after an attack of malaria is used only with regard to blood that is to be fractionated and not for whole blood."—*Mark Falcon Lesses, M.D.*

(Recent inquiry to a Red Cross Regional Blood Center brought the following reply: "Our manual states that persons who have had malaria or who have received intensive suppressive therapy but have had neither a clinical attack nor therapy in the preceding two (2) years may be accepted for donations to be used as whole blood. Those who have had either a clinical attack or suppressive therapy less than two (2) years but more than six (6) months before may be accepted for fractionation only. We do accept these donors for whole blood under the above rules."—Ed.)

Q. "I have been in correspondence with the American Red Cross and the United States Public Health Service concerning the upper age limits for donors of blood. It is my belief that the setting of the age of 60 is eliminating a very large source of blood in that people aged 60 in these times are more likely to be healthy in view of the considerable extension of life that can be anticipated by people at that age over what it was a short time ago.

I would appreciate your information concerning the standards for blood banks published by your organization and your comments on the setting of an absolute standard of 60 beyond which blood will not be acceptable."—*M.D., Sacramento, California.*

A. "The Standards for a Blood Transfusion Service of the Joint Blood Council and the American Association of Blood Banks does not set an absolutely standard age of 60, beyond which blood donors will not be acceptable. The precise wording of the 'Standards' is as follows: 'The written consent of a physician shall be required when the donor is over 60 years of age.' This should be interpreted to mean that a donor over 60 years of age is entirely acceptable if a physician thinks that he is and if he is willing to put it in writing. In many blood banks, donors over the age of 60 are regularly accepted, if this condition is met.

Parenthetically, however, it should be pointed out that only a very small part of the available donor pool between the ages of 21 and 60 is utilized in blood banks, so that there is not too great a need for pushing up the age limits."—*Mark Falcon Lesses, M.D.*

Q. "I operate an approved clinical medical laboratory and for transfusions do the following tests on donor blood: blood type, blood Rh studies, serology, malaria, typhoid and para-

typhoid agglutinations, undulant agglutination and blood count. From a medico-legal standpoint do you consider the above unnecessary?

What tests do you recommend on donor blood?"—*M.D., St. Joseph, Mo.*

A. "The essential tests to perform on donor blood are as follows:

1. Hemoglobin
2. Blood group and Rh; if Rh is apparently negative, another test to exclude Rh_0 variants or Du's should be performed.
3. Serological test to exclude syphilis.

These are the essential tests to perform on donor blood; testing for malaria, typhoid, undulant fever, and a blood count other than the hemoglobin are not required and in fact might lead to a false sense of security. An adequate donor history and examination as outlined in the Standards for a Blood Transfusion Service should aid in the selection of the most suitable donors for blood donation." — *Mark Falcon Lesses, M.D.*

Crossmatch

Q. "Is it permissible and practiced by hospitals in cases of Rh sensitivation of high titer to select a donor by performing a crossmatch with the mother's serum only prior to the baby's birth and then actually give this blood in exchange transfusion without actually crossmatching the baby's blood with the so selected donor? Provided of course Witebsky solution has been added if indicated.

In cases of multiple transfusions when would you recommend to crossmatch the donors with each other? Is this commonly practiced?"—*M.T., A.S.C.P., Staten Island, N. Y.*

A. "When performing a crossmatch for exchange transfusion of a new-born baby with erythroblastosis caused by Rh sensitization, it is most important to crossmatch the intended donor blood with the mother's serum rather than with the baby's blood, because whatever antibodies are present in the baby will be present even more strongly in the mother's serum; in fact, at times, they may not at all be detectable in the baby's serum since they may have coated all of the baby's cells, or be present in the baby's tissues. The addition of Witebsky's substances does not necessarily neutralize immune anti A or anti B. In case of the unavailability of the mother's blood, the serum of the infant must be used for the crossmatch. Naturally all crossmatches should be by sensitive methods, including the indirect Coombs'. For reference I would suggest the book 'Erythroblastosis Fetalis' by Diamond and Allen.

Regarding your second question, in multiple transfusions, crossmatching donors with each other is not recommended. However, when the number of transfusions to be given approaches an exchange transfusion, and for an adult, this would be eight or more transfusions, then measures must be taken to insure compatibility amongst the various units of transfused blood since the serum of the recipient would become for a short time a "foreign" serum rather than that of the recipient. A simple method of assuring compatibility is to perform an adequate antibody screen against a panel of test cells of all the donors and to do the same for the recipient. A more complicated way of achieving the same result is to utilize pools of donor cells and serums against each other. This has been described by Allen in a recent issue of the *Bulletin* of the American Association of Blood Banks."—*Mark Falcon Lesses, M.D.*

Q. "I have two problems I would like to ask your help with. One is to establish a plasma bank. Could you send me information about the best methods for preparing and pooling plasma for use in disaster planning?

The second problem is in regard to crossmatch procedures. On occasion we have had crossmatches which show agglutination on the minor side, only by the Coombs technic. Question: Can this discrepancy be ignored in ordinary transfusion practice or should it be considered significant?"—*M.D., Danville, Va.*

A. "The preparation of plasma is described in detail in the Technical Methods and Procedures of the AABB (page 46-47). A copy of this manual can be ordered from the Central Office of the AABB.

A positive Coombs test on the minor side of the crossmatch which is negative by other technics may indicate the presence of antibodies such as anti-Kell, anti-Fy^a, anti-Jk^a or others in the donor serum. The donor serum should be investigated for specificity of the antibody and not used for recipients with the corresponding antigen in their red cells.

It should be remembered that in all cases where the patient's red cells give a positive direct Coombs test the minor crossmatch will also present positive results when the Coombs technic is applied."—*Morten Grove-Rasmussen, M.D.*